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54 Antibiotic A 42867 derivative.

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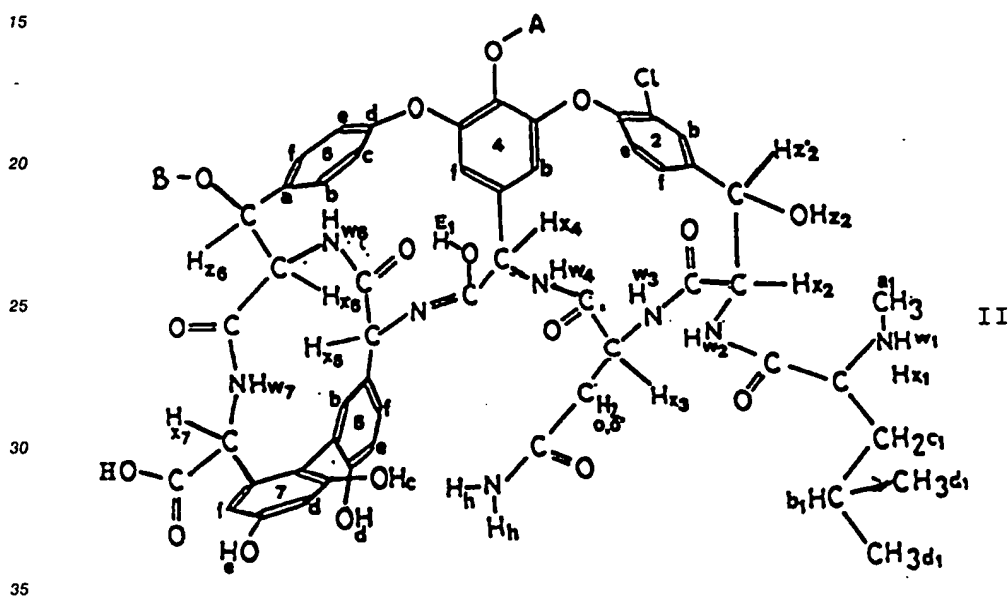
## Description

The present invention is directed to a new antibiotic substance denominated antibiotic A 42867 pseudoaglycon, the pharmaceutically acceptable salts thereof, a process for its preparation from antibiotic A 42867 and pharmaceutical compositions containing the new substance of the invention.

Antibiotic A 42867 pseudoaglycon and the pharmaceutically acceptable salts thereof are particularly active against gram positive bacteria.

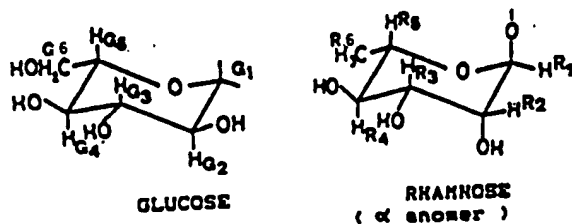
Antibiotic A 42867, which is the starting material for the preparation of the compound of the invention, is an antibiotic substance produced by a *Nocardia* strain, *Nocardia* sp. ATCC 53492, which was deposited on 23 May 1986 under the provisions of the Budapest Treaty. It was described in European Patent Application Publication No.254999.

On the basis of the physico-chemical data available and by reference to the structure of known substances the following formula can be attributed to antibiotic A 42867



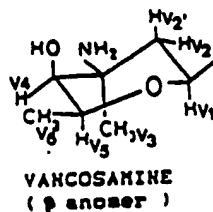
wherein

A represents a disaccharide residue of one unit of glucose and one unit of d-rhamnose, of formula, respectively:



and

B represents a beta-vancosamine unit of formula



As evident to the man skilled in the art, the peptidic bond connecting the moiety containing ring 4 with that containing ring 5 may exist in a tautomeric form (-CO-NH-). In the present disclosure, the above formula I is thus intended to cover also its tautomers.

15 Physico-chemical characteristics of antibiotic A 42867 pseudoaglycon:

A) ultraviolet absorption spectrum which is reported in Figure 1 and exhibits the following absorption maxima:

20

	Lambda max (nm)
a) 0.1 N HCl	279
b) Water	279 305 (shoulder)
c) phosphate buffer pH 7.4	279
d) 0.1 N KOH	299 265 (shoulder)

25

30 B) infrared absorption spectrum in nujol mull which is shown in Figure 2 of the accompanying drawings and exhibits the following absorption maxima (cm<sup>-1</sup>):

3700-3100, 3000-2800 (nujol); 1650; 1590; 1460 (nujol); 1375 (nujol); 1305; 1240; 1210; 1160; 1130; 1060; 1010; 950; 870; 835; 720 (nujol)

35 C) <sup>1</sup>H-NMR spectrum which is shown in Figure 3 and exhibits the following groups of signals (in ppm) at 250 MHz (Bruker Instruments) recorded in DMSO-d<sub>6</sub> (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), (delta = ppm):

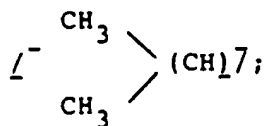
Sugar moiety:

1.22, d [CH<sub>3</sub>-(CH)]; 1.50, s [CH<sub>3</sub>-C(NH<sub>2</sub>)]; 2.25, m [CH<sub>2</sub>-(CH)]; 3.60, m [CH-(CH<sub>3</sub>)]; 4.90, d (anomeric proton)

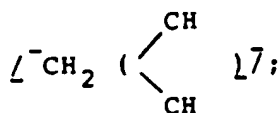
Core moiety:

0.90, d and 0.94 d

40



50 1.74, m [CH(CH<sub>3</sub>)<sub>2</sub>];  
1.60, m



2.32, s [ $\text{CH}_3\text{-(NH)}$ ]; 2.49, s (solvent  $\text{DMSO-d}_5$ ); 3.35, br [ $\text{H}_2\text{O}$ ]; 4.23-6.35 [aromatic and peptidic  $\text{CH}'\text{s}$ ]; 6.35-9.50 [aromatic  $\text{CH}'\text{s}$ , peptidic  $\text{NH}'\text{s}$  and phenolic  $\text{OH}'\text{s}$ ]

s = singlet; d = doublet; m = multiplet; br = broad

D) retention time ( $R_t$ ) of 2.88 relative to antibiotic A 42867 ( $R_t = 8.45$  min) when analyzed by reverse phase HPLC under the following conditions:

column: Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: 3 cm Brownlee Labs RP 18 (5 micrometer)

eluent:

A) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 98:2 (v/v) adjusted to pH 6.0

B) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 30:70 (v/v) adjusted to pH 6.0

Elution mode: linear gradient from 5% to 60% of B in A in 40 min

flow rate: 1.8 ml/min

U.V. detector: 254 nm

internal standard: antibiotic A 42867 ( $R_t = 8.45$  min)

E) elemental analysis showing the presence of one chlorine atom per molecule

F) 2D  $^1\text{H-NMR}$  NOESYPH analysis showing that the molecular chlorine atom is on ring 2 and not on ring 6, on the basis of Nuclear Overhauser effect (dipolar coupling through space) and scalar coupling through chemical bond, in fact:

a) on the basis of scalar coupling, proton 2b (see formula I) shows only a meta coupling with proton 2f but no ortho coupling; (proton 2b is on ring 2 and not on ring 6 on the basis of the following dipolar coupling:

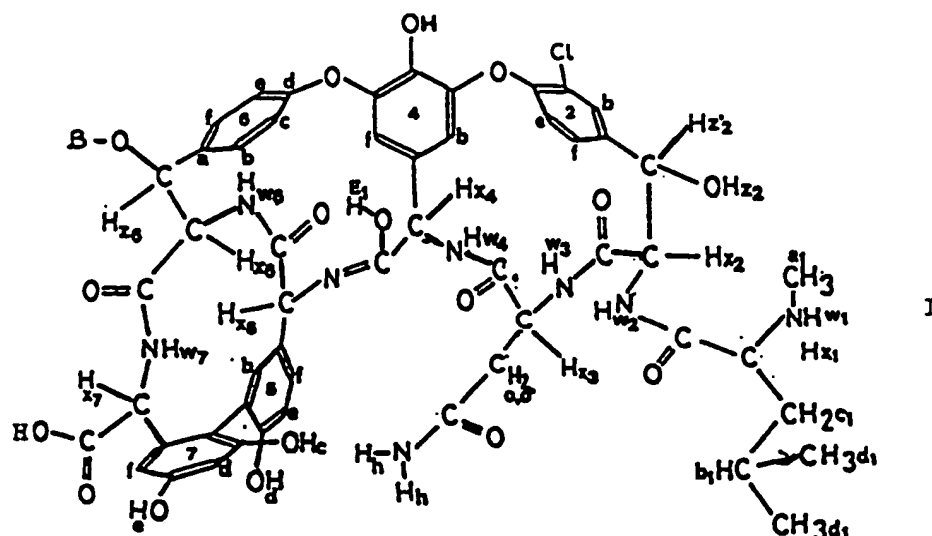
$2b \rightarrow z'_2$ ;  $2b \rightarrow (z_2)\text{OH}$ ;  $(z_2)\text{OH} \rightarrow z'_2$ ;  $z'_2 \rightarrow x_2$ )

b) on the basis of scalar coupling, proton 6b (see formula I) shows an ortho coupling with proton 6c (dipolar coupling  $6b \rightarrow 6c$ ;  $z_6 \rightarrow 6b$ ;  $z_6 \rightarrow x_6$ )

G) a fast atom bombardment (FAB) mass spectrum with  $M + H^+$  at about 1251 (the theoretical cluster ion peaks for the formula  $\text{C}_{60}\text{H}_5\text{N}_9\text{O}_{19}\text{Cl} + \text{H}$  range from 1250 to 1255, average value 1251.68). The FAB spectrum was obtained with a VG 70-250 instrument, bombarding gas Xenon; beam energy 8 KeV; accelerating voltage 6kV; matrix thioglycerol-glycerol, 2:1.

H) acid and basic functions capable of forming salts.

On the basis of the above data and by reference to the structure of known antibiotics, it can tentatively be attributed to the following formula:



wherein B is as defined above.

In view of their similarities, in the present description and claims, when dealing with the biological properties of the compound of the invention, also its internal salt as well as its basic and acid addition salts are intended.

Antibiotic A 42867 pseudoaglycon is prepared from antibiotic A 42867 by controlled acid hydrolysis, conducted in an organic solvent in the presence of a strong acid.

The reaction temperature is preferably between 40 °C and 110 °C and most preferably between 80 °C and 100 °C, with about 90 °C being the most preferred reaction temperature.

5 The reaction time varies depending on the specific reaction conditions.

Generally, the reaction time is between 1 h and 120 h.

However, since the reaction course may be monitored by TLC or HPLC, the skilled man is capable of deciding when the hydrolysis of the starting materials is to be considered as completed and the recovery procedure may be started.

10 Representative examples of strong acids are mineral or organic strong acids such as hydrogen halides, e.g. hydrogen chloride, bromide and iodide, phosphoric acid, sulfuric acid, haloacetic acids, e.g. trichloroacetic acid, trifluoroacetic acid, chlorodifluoroacetic acid and the like.

Suitable organic solvents are such that:

a) they may at least partially solubilize the starting materials;

15 b) the products, once obtained, either separate or may be separated from them according to usual techniques, and

c) in any case, they do not unfavorably interfere with the reaction course.

Examples of said organic solvents are protic or aprotic solvents such as (C<sub>1</sub>-C<sub>4</sub>)alkyl sulfoxides, e.g. dimethylsulfoxide and diethylsulfoxide, (C<sub>1</sub>-C<sub>4</sub>)alkyl formamides, e.g. dimethylformamide, diethylformamide, 20 dioxane, tetrahydrofuran and similar solvents, which are of course compatible with the selected acid.

In general, the hydrolysis is conducted in the presence of a limited amount of water, e.g. from 0.1 to 10% (w/w) of the reaction mixture. This amount of water can obviously be already present either in the starting materials, solvents and/or reagents, or may be added *ad hoc*, if necessary.

A preferred embodiment of the process of the invention is represented by the use of a mixture 25 dimethylsulfoxide/diluted hydrochloric acid at a temperature between 80 °C and 100 °C. Typically, the ratio of the mixture dimethylsulfoxide/diluted hydrochloric acid is from 8:2 to 9.5:0.5. The preferred acid concentration is 0.1 N hydrochloric acid.

Antibiotic A 42867 pseudoaglycon obtained according to the above procedure may be further purified, if necessary or required, according to known per se techniques and preferably by chromatography such as 30 liquid/liquid chromatography, flash chromatography, high pressure liquid chromatography and affinity chromatography.

When affinity chromatography is used, a preferred adsorbent is an immobilized D-Alanyl-D-Alanine as described in EP-A- 122969. Particularly preferred is agarose-epsilon-aminocaproyl-D-Alanyl-D-Alanine. The elution mixture is a mixture of an aqueous buffer and a saline solution or an aqueous base.

35 A preferred eluent is a solution of a volatile base such as aqueous ammonia and most preferably is about 1.5% aqueous ammonia, while a preferred rinsing solution is phosphate buffer pH 6.0.

Alternatively, antibiotic A 42867 pseudoaglycon may be further purified by means of strong or weak anion exchange resins including functionalized polystyrene, acrylic or polydextrane matrices. Examples of weak anion exchange resins are those sold under the following trade-names: Dowex MWA-1 or WGR (Dow 40 Chemical), Amberlite IRA-73 (Rohm and Haas), DEAE-Sephadex (Pharmacia). Examples of strong anion exchange resins which may be used according to invention include those sold under the following trade names: Dowex MSA-1, SBR, SBR-P (Dow Chemical), Amberlite IR-904 (Rohm and Haas) and QAE-Sephadex (Pharmacia).

The elution of the antibiotic substance from these resins is conducted by means of linear gradient 45 mixtures of aqueous solution of electrolytes, such as sodium or potassium hydrochloride, in water or mixtures of water and an organic water-miscible solvent such as a lower alcohol (e. g. (C<sub>1</sub>-C<sub>4</sub>)alkanol) or a lower alkyl ketone (e.g. acetone, methylethyl ketone, etc.)

If further purification is desired or necessary, it can be conveniently achieved by preparative HPLC.

A reverse phase silica gel is in this case used as the adsorbent while the mobile phase is one of those 50 conventionally used in this field, such as a mixture of an aqueous buffer and a polar organic solvent. A preferred aqueous buffer is a sodium phosphate buffer, while a preferred polar organic solvent which can be conveniently used is acetonitrile.

Antibiotic A 42867 pseudoaglycon possesses acid and basic functions and besides forming internal salts, under proper pH conditions, can form salts with organic and inorganic counter-ions according to 55 conventional procedures.

Representative and suitable acid addition salts of the compounds of the invention include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, trichloroacetic, succinic, citric, ascorbic, lactic,

maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic and the like acids.

Representative examples of these bases are: alkali metal or alkaline-earth metal hydroxide such as sodium, potassium, calcium, magnesium, barium hydroxide; ammonia and aliphatic, alicyclic or aromatic organic amines such as methylamine, dimethylamine, trimethylamine, and picoline.

The transformation of the "non-salt" compounds of the invention into the corresponding addition salts, and the reverse, i.e. the transformation of an addition salt of a compound of the invention into the non-salt form, are within the ordinary technical skill and are encompassed by the present invention.

For instance antibiotic A 42867 pseudoaglycon can be transformed into the corresponding acid addition-salt by dissolving the non-salt form in an aqueous solvent and adding a slight molar excess of the selected acid or base. The resulting solution or suspension is then lyophilized to recover the desired salt.

In case the final salt is insoluble in a solvent where the non-salt form is soluble, it is recovered by filtration from the organic solution of the non-salt form after addition of the stoichiometric amount or a slight molar excess of the selected acid or base.

The non-salt form can be prepared from a corresponding acid or base salt dissolved in an aqueous solvent which is then neutralized to free the non-salt form.

When following the neutralization the elimination of the excess of acid or base is necessary, a common desalting procedure may be employed.

For example, column chromatography on silanized silica gel, non-functionalized polystyrene, acrylic and controlled pore polydextrane resins (such as Sephadex® LH 20) or activated carbon may be conveniently used. After eluting the undesired salts with an aqueous solution, the desired product is eluted by means of a linear gradient or a step-gradient of a mixture of water and a polar or apolar organic solvent, such as acetonitrile/water from 50:50 to about 100% acetonitrile.

As it is known in the art, the salt formation either with pharmaceutically acceptable acids (or bases) or non-pharmaceutically acceptable acids (or bases) may be used as a convenient purification technique. After formation and isolation, the salt form of antibiotic A 42867 pseudoaglycon can be transformed into the corresponding non-salt form or into a pharmaceutically acceptable salt form.

The antibacterial activity of the compound of the invention can be demonstrated *in vitro* by means of standard dilution tests on different microorganism cultures.

Culture media and growth conditions for MIC (minimal inhibitory concentration) determinations were as follows: Isosensitest broth (Oxoid), 24 h, for staphylococci, *Strep. faecalis* and Gram-negative bacteria (*Escherichia coli*); Todd-Hewitt broth (Difco), 24 h for other streptococcal species; GC base broth (Difco) + 1% Isovitalex (BBL), 48 h, CO<sub>2</sub>-enriched atmosphere for *Neisseria gonorrhoeae*; Brain Heart broth (Difco) + 1% Supplement C (Difco), 48 h for *Haemophilus influenzae*. PPLO broth with supplements as in R.T. Evans and D. Taylor-Robinson (J. Antimicrob. Chemother. 4, 57), 24 h for *U. urealyticum*. Incubation was at 37 °C. Inocula were as follows: about 10<sup>4</sup> color-changing units/ml for *U. urealyticum*; about 10<sup>4</sup>-10<sup>5</sup> colony-forming units/ml for other broth dilution MICs.

The minimal inhibitory concentrations (MIC, microgram/ml) for some microorganisms are reported below in Table I.

TABLE I

Strain	M.I.C. (microgram/ml) Antibiotic A 42867 pseudoaglycon
<u>Staph. aureus</u> L165 ( $10^4$ cfu/ml)	1
<u>Staph. aureus</u> $10^6$ cfu/ml	1
<u>Staph. aureus</u> 30% bovine serum	2
<u>Staph. epidermidis</u> L147 ATCC 12228 (coagulase negative)	2
<u>Staph. haemolyticus</u> clin isolate	2
<u>Strep. pyogenes</u> L49 C203	0.5
<u>Strep. pneumoniae</u> L44 UC41	1
<u>Strep. faecalis</u> L149 ATCC 7080	2
<u>Strep. mitis</u> L796 (clinical isolate)	0.5
<u>Neisseria gonorrhoeae</u> L997 ISM68/126	64
<u>Haemophilus influenzae</u> type b L 970 ATCC 19418	>128
<u>Ureaplasma urealyticum</u> 1479 clin. isolate	>128
<u>Escherichia coli</u> L47 SKF 12140	>128

The antimicrobial activity of the compounds of the invention is confirmed also in experimental septicemia in the mouse.

- 55 Control and treatment groups contain ten CD-1 mice (Charles River) weighing 18-22 g. They are infected intraperitoneally with 0.5 ml of bacterial suspension prepared by diluting an overnight culture of S. pyogenes C 203 (L 49) with sterile peptonized saline. Inocula are adjusted so that untreated animals died of septicemia within 48 h. The compounds to be tested are administered subcutaneously immediately after

infection. On the 7th day, the ED<sub>50</sub> in mg/kg is calculated by the method of Spearman and Kärber (D.J. Finney "Statistical Methods in Biological Assay", Griffin, page 524, 1952) from the percentage of surviving animals at each dose.

In general, for antibacterial treatment antibiotic A 42867 pseudoaglycon as well as the non-toxic pharmaceutically acceptable salts thereof or mixture thereof, can be administered by different routes such as topically or parenterally. The parenteral administration is, in general, the preferred route of administration.

Compositions for injection may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain adjuvants such as suspending, stabilizing and/or dispersing agents.

Alternatively, the active ingredient may be in powder form for reconstitution at the time of delivery when a suitable vehicle, such as sterile water, is added thereto.

Depending on the route of administration, these compounds can be formulated into various dosage forms.

In some instances, it may be possible to formulate the compounds of the invention in enteric-coated dosage forms for oral administration which may be prepared as known in the art (see for instance "Remington's Pharmaceutical Sciences", fifteenth edition, Mack Publishing Company, Easton, Pennsylvania, USA, page 1614).

This could be especially the case when the absorption of the antimicrobial substance in the enteric tract is particularly desired while passing unaltered through the gastric tract.

The amount of active principle to be administered depends on various factors such as the size and condition of the subject to be treated, the route and frequency of administration, and the causative agent involved.

The antibiotic substances of the present invention and the physiologically acceptable salts thereof, are generally effective at a daily dosage of between about 0.5 and 50 mg of active ingredient per kilogram of patient body weight, optionally divided into 1 to 4 administrations per day.

Particularly desirable compositions are those prepared in dosage units containing from about 100 to about 5,000 mg per unit.

Representative examples of vehicles suitable for injection are: sterile water for injection, Ringer's solution, 0.9% saline and 5% dextrose.

For i.v. infusion, the suitable concentration of the antibiotic in the vehicle is between about 5% and 10%.

Other suitable formulations for dosage units are hermetically sealed vials, plastic pouches, sterile, rubber-stoppered vials and the like.

In addition, the antibiotic substance of the invention can be formulated in a topical preparation such as a solution, a cream or a lotion. These preparations conveniently contain from 0.1 to 15% (w/v) of the active ingredient.

Besides its activity as medicament, antibiotic A 42867 pseudoaglycon, or an acceptable salt thereof, can be used as an animal growth promoter.

For this purpose, a compound of the invention is administered orally in a suitable feed. The exact concentration employed is that which is required to provide for the active agent in a growth promotant effective amount when normal amounts of feed are consumed.

The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration.

Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed.

The way in which such feed premixes and complete rations can be prepared and administered is described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., S. Francisco, USA, 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Oregon, USA, 1977) which are incorporated herein by reference.



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using reverse-phase silanized silica gel plates (RA-18 F<sub>254</sub>)

Visualization:

- U.V. light at 254 nm
- Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))
- Bioautography using B. subtilis ATCC 6633 on minimal Davis medium.

H) MW of about 1559 desumed from a FAB-MS spectrum showing the  $M + H^+$  peak at 1560.

Brief description of the drawings:

Figure 1 relates to the U.V. spectrum of antibiotic A 42867 pseudoaglycon under the conditions reported above.

In particular, the symbol:

----- refers to the assay in 0.1 N HCl

15



refers to the assay in water

20



refers to the assay in phosphate buffer pH 7.4

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refers to the assay in 0.1 N KOH

Figure 2 relates to the I.R. absorption spectrum of antibiotic A 42867 pseudoaglycon under the conditions reported above.

Figure 3 relates to the  $^1\text{H-NMR}$  spectrum of antibiotic A 42867 pseudoaglycon under the conditions reported above.

Figure 4 relates to the  $^1\text{H-NMR}$  spectrum of antibiotic A 42867 under the conditions reported above.

The letters over each signal represent the attribution given to it.

Preparation 1

Production of antibiotic A 42867

40

The stock culture of the producing organism (Nocardia sp. ATCC 53492) is streaked on oatmeal agar slants and incubated at 28 °C for 2 weeks.

One loopful of strain growth is inoculated into a 500 ml Erlenmeyer flask containing 100 ml of a seed medium composed of dextrose 2.0%, soybean meal 0.8%, yeast extract 0.2%, NaCl 0.1% and  $\text{CaCO}_3$  0.4% whose pH of the medium has been adjusted to 7.3 before sterilization.

45

The flask is incubated on a rotary shaker at 28 °C for 72 hours. A 100 ml aliquot of the culture is then inoculated into a jar fermentor containing 4 liters of the same seed medium and the culture is incubated at 28 °C for 48 hours with agitation of about 900 rpm and aeration of one standard liter of air per volume per minute.

50

After inoculation of 4 liters of the seed culture into a jar fermentor containing 200 liters of fermentation medium having the same composition as the seed medium, fermentation is carried out for 96 hours with agitation of about 250 rpm and aeration of one standard liter of air per volume per minute.

The antibiotic activity was monitored by microbiological assay using B. subtilis cultured on minimal Davis medium.

55

## Preparation 2

### Recovery of antibiotic A 42867

5 The whole fermentation broth (400 liters) obtained as described in Preparation 1 is filtered using a filter aid (Hyflo-FloMa®), on a rotatory filter. The filtered broth is adjusted to pH 7.5 with 2 N hydrochloric acid, and added to 1000 ml of pre-swollen D-Ala-D-Ala-aminocaproyl-Sepharose-4B modified matrix (prepared as described in EP-A- 122969) and left overnight under slight stirring.

10 The resin is recovered by filtration and washed with about 10 l of 0.5% (w/v) HCl-Tris buffer pH 7.5 which contains 5% (w/v) NaCl and then with water (4 x 5 l) while the broth is discharged.

The product selectively bound to the resin is eluted with 1.5% (w/v) ammonia hydroxide (4 x 5 l) and concentrated to a small volume (about 1800 ml) by means of azeotropic distillation with n-butanol under reduced pressure.

The concentrated aqueous solution is lyophilized obtaining crude antibiotic A 42867 (75.6 g).

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## Preparation 3

### Purification of crude antibiotic A 42867

20 Crude antibiotic A 42867 obtained by following the procedure of Preparation 2 (75 g) is dissolved in 2 liters of water containing 2 M sodium chloride, adjusted to pH 7.5 with 0.1 N sodium hydroxide solution, and then filtered.

The filtrate is applied at 500 ml/hour to a 1000 ml column (0.1 x 0.1 m) of pre-swollen D-Ala-D-Ala-ε-aminocaproyl-Sepharose-4B modified matrix (prepared as described in EP-A- 122969) previously equilibrated with 0.04 M borate buffer pH 7.5 containing 2 M sodium chloride and 0.6 ml of Triton® x 100 (Baker grade).

The column is washed with 8 l of 8 M urea (pH 7.5) with flow rate of 500 ml/h followed by 70 l of aqueous NaOH at pH 10 collecting fractions of 1000 ml each.

30 These fractions are assayed on B. subtilis cultures by agar-disc assay and those fractions which are inactive are discharged while those active (like fractions 63-70, in this case) are combined, concentrated to a small volume (500 ml) under reduced pressure by means of azeotropic distillation with n-butanol and lyophilized to give antibiotic A 42867 (4 g).

## Preparation 4

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### Purification and desalination of antibiotic A 42867

3.5 g of antibiotic A 42867 obtained by following the procedure of Preparation 3 is dissolved in 70 ml of a solution of sodium dihydrogen phosphate monohydrate (2.5 g/l) and acetonitrile (91:9) and filtered.

40 10 ml of this filtrate are applied to a stainless steel column (2 x 50 cm) packed with 40 g of 10 micrometer RP 18 Lichrosorb reverse-phase silica gel (Merck).

The column is part of a Chromatospac Modulprep unit (Jobin Yvon, 16-18 Rue de Canal 91169 Longjumeau, France).

45 The column is eluted at 8 ml/min with the same solution used to dissolve the sample and fractions of 50 ml are collected.

Each fraction is monitored by HPLC and paper-disc bioassay on susceptible microorganisms such as B. subtilis.

50 The fractions active on B. subtilis of seven runs are combined, acetonitrile is removed by distillation under reduced pressure and the residue is diluted with a quantity of water which was about the volume of the initial solution.

The solution is adjusted to pH 7.5 and later applied a flow rate of 100 ml/h to a column (5 x 15 cm) of pre-swollen D-Ala-D-Ala-ε-aminocaproyl-Sepharose-4B modified matrix (prepared as described in EP-A- 122969) previously equilibrated with 0.04 M borate buffer pH 7.5.

The column is washed with 8 l of water (acidified with 0.5 ml/l of 1 N hydrochloric acid).

55 The column is then eluted with 1.5% (w/v) ammonia hydroxide collecting fractions of 100 ml each.

Those fractions active against B. subtilis are pooled, concentrated under pressure and lyophilized to give 1.2 g of a desalted preparation of antibiotic A 42867 whose physico-chemical characteristics are reported before.

The following examples further illustrate the invention but should not be interpreted as limiting it in any way.

#### Example 1

##### Preparation of antibiotic A 42867 pseudoaglycon

Antibiotic A 42867 prepared substantially according to Preparation 3 (1 g) is dissolved in a mixture dimethylsulfoxide/1N hydrochloric acid, 9:1 (35 ml) and heated to 85 °C-90 °C.

The reaction course is monitored by HPLC and when the starting materials are about completely reacted (after about 15 h) the reaction is quenched with 0.1 M sodium phosphate buffer pH 7.0 (250 ml). Antibiotic A 42867 pseudoaglycon is separated from this mixture by the following affinity chromatography procedure:

The aqueous mixture obtained above (750 ml) is applied to a Sepharose-D-Alanyl-D-Alanine chromatography column prepared as described in EP-A- 122969 (200 ml of swollen resin in 10 mM TRIS.HCL pH 7.5 buffer; bed height 10 cm).

This column is rinsed with 0.1 M sodium phosphate buffer pH 7.0, monitoring the eluates with U.V. light (254 nm) until no adsorbance is detected. Then the column is eluted with 1.5% (w/v) aqueous ammonia (about 1 l, flow rate 200 ml/h) and the eluate concentrated to a small volume under reduced pressure by azeotropical distillation with n-butanol and lyophilized yielding antibiotic A 42867 pseudoaglycon (349 mg; HPLC purity: 85%).

By repeating this experiment but using a mixture DMSO/1 M HCl 8.5:1.5 at 100 °C for about 10 h or a mixture DMSO/1 M HCl 9.5:0.5 at 90 °C for about 12 h, the compound of the title is obtained.

#### Example 2

##### Further purification of antibiotic A 42867 pseudoaglycon

Antibiotic A 42867 pseudoaglycon obtained (250 mg) is dissolved in a mixture of sodium dihydrogen phosphate monohydrate (2.5 g/l) and acetonitrile, 92:8, adjusted to pH 6 (5 ml).

This solution (1 ml) is applied to a HPLC semi-preparative pre-packed column (250 x 10 mm (i.d.), Merck, packed with reverse phase silica gel Lichrosorb RP 18, 7 micrometer) which is then eluted with a flow rate of 4 ml/min of a mixture of phase A and phase B with a linear gradient from 5% to 50% of phase B in A.

Phase A) (2.5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN 98:2 (v/v) adjusted to pH 6.0 with NaOH.

Phase B) (2.5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN 30:70 (v/v) adjusted to pH 6.0 with NaOH.

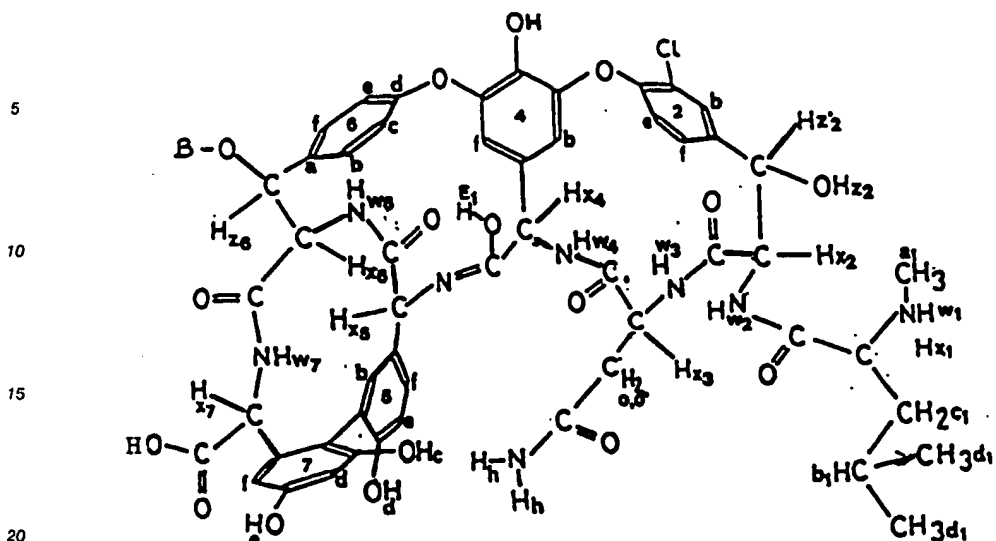
The eluates are U.V. monitored at 254 nm and the eluted fractions with homogeneous content are pooled.

The eluates containing the purified antibiotic A 42867 pseudoaglycon of 5 subsequent chromatographic runs are pooled and desalted as usual by loading them on a column of 50 ml swollen sepharose-D-Ala-D-Ala. After removing the salts with 100 ml of 1 mM HCl, the antibiotic is eluted with 3 x 100 ml of 1.5% (w/v) aqueous ammonia. The ammonia eluates are then collected and concentrated under reduced pressure yielding 160 mg of pure antibiotic A 42867 pseudoaglycon.

#### Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Antibiotic A 42867 pseudoaglycon and the pharmaceutically acceptable salts thereof having the following formula (unsalified compound):



wherein

B represents a beta-vancosamine unit of formula



and the tautomers thereof.

2. Antibiotic A 42867 pseudoaglycon and the pharmaceutically acceptable salts thereof having the following physico-chemical parameters (unsalified form):

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

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50

	Lambda max (nm)
a) 0.1 N HCl	279
b) Water	279 305 (shoulder)
c) phosphate buffer pH 7.4	279
d) 0.1 N KOH	299 265 (shoulder)

B) infrared absorption spectrum in nujol mull which exhibits the following absorption maxima ( $\text{cm}^{-1}$ ):  
 3700-3100, 3000-2800 (nujol); 1650; 1590; 1460 (nujol); 1375 (nujol); 1305; 1240; 1210; 1160;  
 1130; 1060; 1010; 950; 870; 835; 720 (nujol)

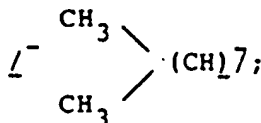
C)  $^1\text{H}$ -NMR spectrum which exhibits the following groups of signals (in ppm) at 250 MHz (Bruker Instruments) recorded in  $\text{DMSO-}d_6$  (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), ( $\delta$  = ppm):

Sugar moiety:

1.22, d [ $\text{CH}_3\text{-(CH)}$ ]; 1.50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ]; 2.25, m [ $\text{CH}_2\text{-(CH)}$ ]; 3.60, m [ $\text{CH-(CH}_3\text{)}$ ]; 4.90, d (anomeric proton)

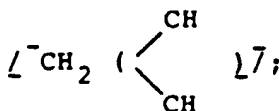
Core moiety:

0.90, d and 0.94 d



1.74, m [ $\text{CH(CH}_3\text{)}_2$ ];

1.60, m



2.32, s [ $\text{CH}_3\text{-(NH)}$ ]; 2.49, s (solvent  $\text{DMSO-}d_6$ ); 3.35, br [ $\text{H}_2\text{O}$ ]; 4.23-6.35 [aromatic and peptidic  $\text{CH}$ 's]; 6.35-9.50 [aromatic  $\text{CH}$ 's, peptidic  $\text{NH}$ 's and phenolic  $\text{OH}$ 's]

s = singlet; d = doublet; m = multiplet; br = broad

D) retention time ( $R_t$ ) of 2.88 relative to antibiotic A 42867 ( $R_t$  = 8.45 min) when analyzed by reverse phase HPLC under the following conditions:

column: Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: 3 cm Brownlee Labs RP 18 (5 micrometer)

eluents:

A) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 98:2 (v/v) adjusted to pH 6.0

B) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 30:70 (v/v) adjusted to pH 6.0

Elution mode: linear gradient from 5% to 60% of B in A in 40 min

flow rate: 1.8 ml/min

U.V. detector: 254 nm

internal standard: antibiotic A 42867 ( $R_t$  = 8.45 min)

E) elemental analysis showing the presence of one chlorine atom per molecule

F) 2D  $^1\text{H}$ -NMR NOESYPH analysis showing that the molecular chlorine atom is on ring 2 and not on ring 6, on the basis of Nuclear Overhauser effect (dipolar coupling through space) and scalar coupling through chemical bond, in fact:

a) on the basis of scalar coupling, proton 2b (see formula I) shows only a meta coupling with proton 2f but no ortho coupling; (proton 2b is on ring 2 and not on ring 6 on the basis of the following dipolar coupling:

$2b \rightarrow z'_2$ ;  $2b \rightarrow (z_2)\text{OH}$ ;  $(z_2)\text{OH} \rightarrow z'_2$ ;  $z'_2 \rightarrow x_2$ )

b) on the basis of scalar coupling, proton 6b (see formula I) shows an ortho coupling with proton 6c (dipolar coupling  $6b \rightarrow 6c$ ;  $z_6 \rightarrow 6b$ ;  $z_6 \rightarrow x_6$ )

G) a fast atom bombardment (FAB) mass spectrum with  $M + H^+$  at about 1251 (the theoretical cluster ion peaks for the formula  $\text{C}_{60}\text{H}_{64}\text{N}_9\text{O}_{19}\text{Cl} + \text{H}$  range from 1250 to 1255, average value 1251.68). The FAB spectrum was obtained with a VG 70-250 instrument, bombarding gas Xenon; beam energy 8 KeV; accelerating voltage 6kV; matrix thioglycerol-glycerol, 2:1.

H) acid and basic functions capable of forming salts.

3. A process for preparing a compound of claim 1 or 2 which comprises reacting a compound of formula II



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- 15

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	Lambda max (nm)
a) 0.1 N HCl	282
b) Water	282
c) phosphate buffer pH 7.4	282
d) phosphate buffer pH 9	282 305 (shoulder)
e) 0.1 N KOH	305 265 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima ( $\text{cm}^{-1}$ ):  
3700-3100, 3000-2800 (nujol); 1650; 1580; 1460 (nujol) 1375 (nujol); 1300; 1235; 1210; 1160; 1130;  
1060; 1025; 1000; 970; 840; 790-700; 720 (nujol)

C)  $^1\text{H}$ -NMR spectrum which exhibits the following groups of signals (in ppm) at 500 MHz recorded in DMSO- $\text{d}_6$  (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), ( $\delta$  = ppm):

$\text{d}_1$  0.90;  $\text{R}_6$  1.06;  $\text{V}_6$  1.23;  $\text{V}_3$  1.52;  $\text{c}_1$  1.62;  $\text{b}_1$  1.76;  $\text{V}_2, \text{V}_2'$  2.30;  $\text{a}_1$  (N- $\text{CH}_3$ ) 2.38;  $\text{R}_4$  3.12;  $\text{x}_1, \text{R}_3, \text{V}_4$  3.10-3.50;  $\text{V}_5$  3.60;  $\text{R}_2$  3.79;  $\text{x}_6, \text{R}_5$  4.22;  $\text{x}_7$  4.51;  $\text{x}_5$  4.75;  $\text{V}_1$  4.88,  $\text{R}_1$  4.96;  $\text{x}_2$  5.02;  $\text{x}_3, \text{z}_6$  5.12;  $\text{z}'_2$  5.22;  $4\text{f}$  5.33;  $4\text{b}$  5.53;  $\text{Z}_2(\text{OH})$  5.88;  $7\text{f}$  6.23;  $\text{x}_4$  6.34;  $7\text{d}$  6.41;  $\text{h}$  6.62;  $5\text{c}, \text{w}_3$  6.76;  $5\text{f}$  6.84;  $6\text{c}$  7.12;  $5\text{b}$  7.15;  $2\text{b}$  7.26;  $\text{w}_6, \text{h}$  7.32;  $6\text{b}$  7.50;  $\text{w}_4$  8.15;  $\text{w}_7$  8.45;  $7\text{c}$  9.10;  $5\text{d}, \text{e}_1$  9.32;  $7\text{e}$  9.39;

D) retention-time ( $\text{R}_t$ ) of 0.665 relative to Vancomycin.HCl (Vancocin, Eli Lilly,  $\text{R}_t$  = 9.96 min) when analyzed by reverse phase HPLC under the following conditions:

column: Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: Brownlee Labs RP 18 (5 micrometer)

eluent A:

$\text{CH}_3\text{CN}$  2% } adjusted at  
(2.5 g/l)  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  98% } pH 6.0

eluent B:

$\text{CH}_3\text{CN}$  70% } adjusted at  
(2.5 g/l)  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  30% } pH 6.0

elution: linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate: 1.6 ml/min

U.V. detector: 254 nm

internal standard: Vancomycin.HCl ( $\text{R}_t$  = 9.96 min) (Vancocin, Eli Lilly)

E) elemental analysis, after the sample has been previously dried at about  $140^\circ\text{C}$  under inert atmosphere which indicates the following approximate percentage composition (average): carbon 53.3%; hydrogen 5.9%; nitrogen 7.85%; chlorine (total) 4.41%; chlorine (ionic) 2.22%. Inorganic residue at  $900^\circ\text{C}$  in the air: 0.875%.

F) acid-base titration profile in water upon titration with 0.05N aqueous KOH of a sample previously added with excess of aqueous HCl which shows  $\text{pK}_a$  values at 3.2, 7.1 and 8.3

G)  $\text{R}_f$  value of 0.56 in the following chromatographic system:

a mixture of aqueous NaCl (87.5 g/l):aqueous  $\text{NaH}_2\text{PO}_4$  (0.5 g/l), and acetonitrile, 70:30, adjusted to pH 6.0

using reverse-phase silanized silica gel plates (RA-18 F<sub>254</sub>)

Visualization:

- U.V. light at 254 nm
- Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))

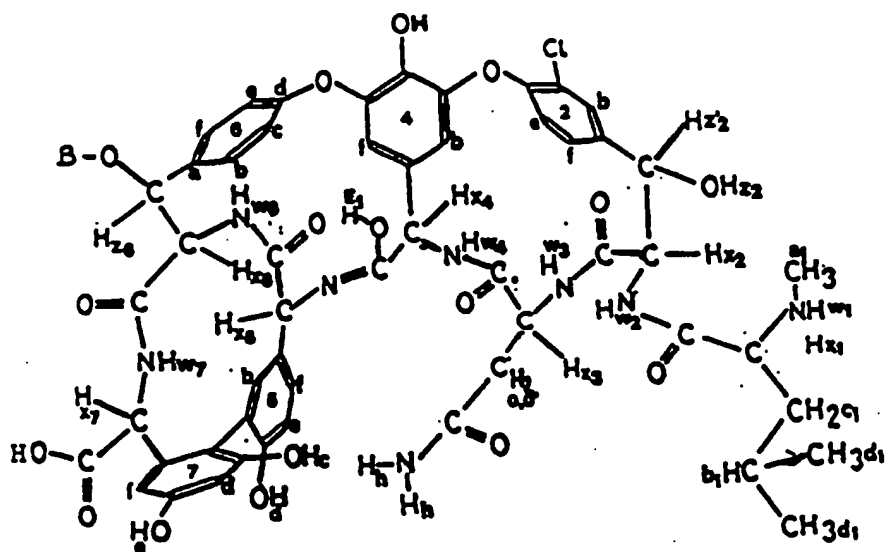


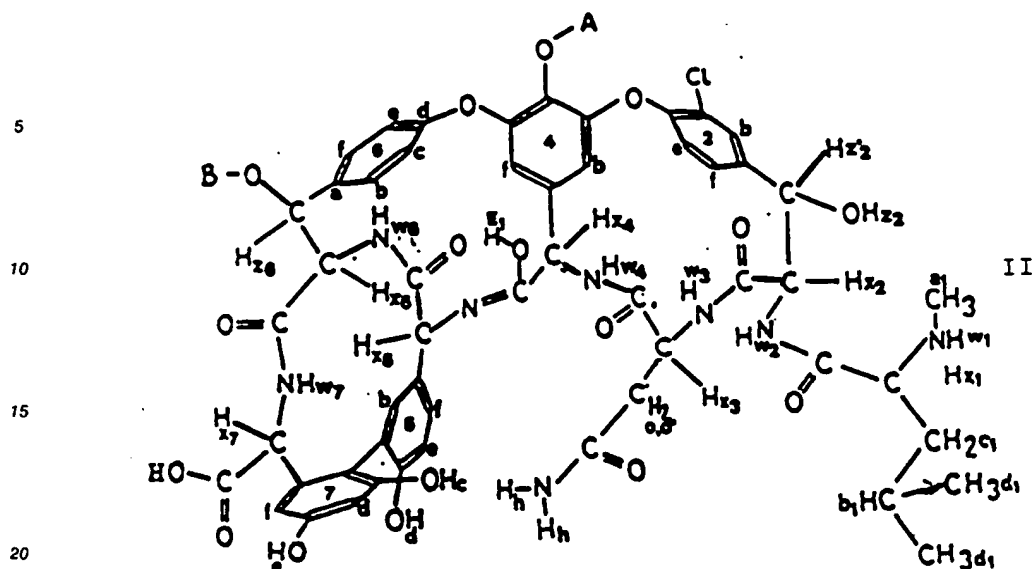
- Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.  
H) MW of about 1559 desumed from a FAB-MS spectrum showing the  $M + H^+$  peak at 1560.

5. A process according to claim 3 wherein the strong acid is hydrochloric acid.
6. A process according to claim 3 wherein the solvent is dimethylsulfoxide or dimethylformamide.
7. A process according to claim 3 wherein the reaction temperature is between 80 °C and 110 °C.
8. Use of a compound of claim 1 or 2 for preparing a medicament for use as an antibiotic.
9. A pharmaceutical composition comprising a compound of claim 1 in admixture with a pharmaceutically acceptable vehicle.
10. A compound of claim 1 or 2 for use as a medicament.

Claims for the following Contracting States : ES, GR

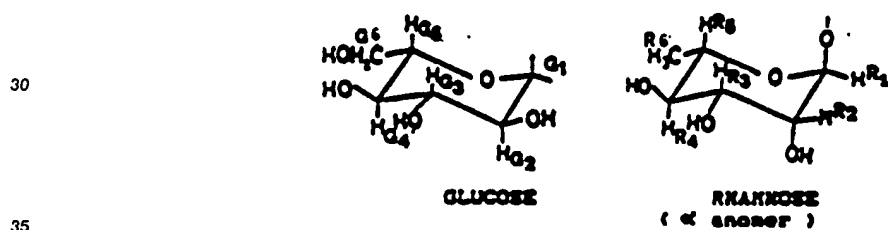
1. A process for preparing antibiotic A 42867 pseudoaglycon and the pharmaceutically acceptable salts thereof having the following formula (unsalified compound):





wherein

A represents a disaccharide residue of one unit of glucose and one unit of d-rhamnose, of formula, respectively:



and

B represents a beta-vancosamine unit of formula



or a tautomer thereof,

under controlled acid hydrolysis in an organic solvent and in the presence of a strong acid.

- 55 2. A process for preparing antibiotic A 42867 pseudoaglycon and the pharmaceutically acceptable salts thereof having the following physico-chemical parameters (unsalified form):

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	Lambda max (nm)
a) 0.1 N HCl	279
b) Water	279 305 (shoulder)
c) phosphate buffer pH 7.4	279
d) 0.1 N KOH	299 265 (shoulder)

B) infrared absorption spectrum in nujol mull which exhibits the following absorption maxima ( $\text{cm}^{-1}$ ):  
3700-3100, 3000-2800 (nujol); 1650; 1590; 1460 (nujol); 1375 (nujol); 1305; 1240; 1210; 1160;  
1130; 1060; 1010; 950; 870; 835; 720 (nujol)

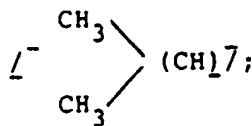
C)  $^1\text{H}$ -NMR spectrum which exhibits the following groups of signals (in ppm) at 250 MHz (Bruker Instruments) recorded in  $\text{DMSO-d}_6$  (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), ( $\delta$  = ppm):

Sugar moiety:

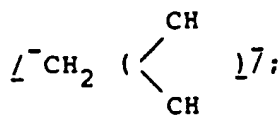
1.22, d [ $\text{CH}_3\text{-(CH)}$ ]; 1.50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ]; 2.25, m [ $\text{CH}_2\text{-(CH)}$ ]; 3.60, m [ $\text{CH-(CH}_3\text{)}$ ]; 4.90, d (anomeric proton)

Core moiety:

0.90, d and 0.94 d



1.74, m [ $\text{CH(CH}_3\text{)}_2$ ];  
1.60, m



2.32, s [ $\text{CH}_3\text{-(NH)}$ ]; 2.49, s (solvent  $\text{DMSO-d}_6$ ); 3.35, br [ $\text{H}_2\text{O}$ ]; 4.23-6.35 [aromatic and peptidic CH's]; 6.35-9.50 [aromatic CH's, peptidic NH's and phenolic OH's]

s = singlet; d = doublet; m = multiplet; br = broad

D) retention time ( $R_t$ ) of 2.88 relative to antibiotic A 42867 ( $R_t$  = 8.45 min) when analyzed by reverse phase HPLC under the following conditions:

column: Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: 3 cm Brownlee Labs RP 18 (5 micrometer)

eluents:

A) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 98:2 (v/v) adjusted to pH 6.0

B) (2.5 g/l)  $\text{NaH}_2\text{PO}_4$  /  $\text{CH}_3\text{CN}$ , 30:70 (v/v) adjusted to pH 6.0

Elution mode: linear gradient from 5% to 60% of B in A in 40 min

flow rate: 1.8 ml/min

U.V. detector: 254 nm

internal standard: antibiotic A 42867 ( $R_t$  = 8.45 min)

E) elemental analysis showing the presence of one chlorine atom per molecule

F) 2D  $^1\text{H}$ -NMR NOESYPH analysis showing that the molecular chlorine atom is on ring 2 and not on ring 6, on the basis of Nuclear Overhauser effect (dipolar coupling through space) and scalar coupling through chemical bond, in fact:

a) on the basis of scalar coupling, proton 2b (see formula I) shows only a meta coupling with proton 2f but no ortho coupling; (proton 2b is on ring 2 and not on ring 6 on the basis of the following dipolar coupling:

$2b \rightarrow z'_2$ ;  $2b \rightarrow (z_2)OH$ ;  $(z_2)OH \rightarrow z'_2$ ;  $z'_2 \rightarrow x_2$ )

b) on the basis of scalar coupling, proton 6b (see formula I) shows an ortho coupling with proton 6c (dipolar coupling  $6b \rightarrow 6c$ ;  $z_6 \rightarrow 6b$ ;  $z_6 \rightarrow x_6$ )

G) a fast atom bombardment (FAB) mass spectrum with  $M + H^+$  at about 1251 (the theoretical cluster ion peaks for the formula  $C_{60}H_{64}N_3O_{19}Cl + H$  range from 1250 to 1255, average value 1251.68). The FAB spectrum was obtained with a VG 70-250 instrument, bombarding gas Xenon; beam energy 8 KeV; accelerating voltage 6kV; matrix thioglycerol-glycerol, 2:1.

H) acid and basic functions capable of forming salts.

3. A process for preparing a compound of claim 1 or 2 which comprises reacting antibiotic A 42867 having the following characteristics:

A) ultraviolet absorption spectrum which exhibits the following absorption maxima:

	Lambda max (nm)
a) 0.1 N HCl	282
b) Water	282
c) phosphate buffer pH 7.4	282
d) phosphate buffer pH 9	282 305 (shoulder)
e) 0.1 N KOH	305 265 (shoulder)

B) infrared absorption spectrum which exhibits the following absorption maxima ( $cm^{-1}$ ):

3700-3100, 3000-2800 (nujol); 1650; 1580; 1460 (nujol); 1375 (nujol); 1300; 1235; 1210; 1160; 1130; 1060; 1025; 1000; 970; 840; 790-700; 720 (nujol)

C)  $^1H$ -NMR spectrum which exhibits the following groups of signals (in ppm) at 500 MHz recorded in  $DMSO-d_6$  (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm), ( $\delta$  = ppm):

$d_1$  0.90;  $R_6$  1.06;  $V_6$  1.23;  $V_3$  1.52;  $c_1$  1.62;  $b_1$  1.76;  $V_2, V_2'$  -2.30;  $a_1(N-CH_3)$  2.38;  $R_4$  3.12;  $x_1, R_3, V_4$  3.10-3.50;  $V_5$  3.60;  $R_2$  3.79;  $x_6, R_5$  4.22;  $x_7$  4.51;  $x_5$  4.75;  $V_1$  4.88;  $R_1$  4.96;  $x_2$  5.02;  $x_3, z_6$  5.12;  $z'_2$  5.22;  $4f$  5.33;  $4b$  5.53;  $z_2(OH)$  5.88;  $7f$  6.23;  $x_4$  6.34;  $7d$  6.41;  $h$  6.62;  $5c, w_3$  6.76;  $5f$  6.84;  $6c$  7.12;  $5b$  7.15;  $2b$  7.26;  $w_6, h$  7.32;  $6b$  7.50;  $w_4$  8.15;  $w_7$  8.45;  $7c$  9.10;  $5d, e_1$  9.32;  $7e$  9.39;

D) retention-time ( $R_t$ ) of 0.665 relative to Vancomycin.HCl (Vancocin, Eli Lilly,  $R_t$  9.96 min) when analyzed by reverse phase HPLC under the following conditions:

column: Ultrasphere ODS (5 micrometer) Altex (Beckman) 4.6 mm (i.d.) x 250 mm

pre-column: Brownlee Labs RP 18 (5 micrometer)

eluent A:

$CH_3CN$  2% } adjusted at  
 (2.5 g/l)  $NaH_2PO_4 \cdot H_2O$  98% } pH 6.0

eluent B:

$CH_3CN$  70% } adjusted at  
 (2.5 g/l)  $NaH_2PO_4 \cdot H_2O$  30% } pH 6.0

elution: linear gradient from 5% to 60% of eluent B in eluent A, in 40 min

flow rate: 1.6 ml/min

U.V. detector: 254 nm

internal standard: Vancomycin.HCl ( $R_f = 9.96$  min) (Vancocin, Eli Lilly)

E) elemental analysis, after the sample has been previously dried at about 140 °C under inert atmosphere which indicates the following approximate percentage composition (average): carbon 53.3%; hydrogen 5.9%; nitrogen 7.85%; chlorine (total) 4.41%; chlorine (ionic) 2.22%. Inorganic residue at 900 °C in the air: 0.875%.

F) acid-base titration profile in water upon titration with 0.05N aqueous KOH of a sample previously added with excess of aqueous HCl which shows pKa values at 3.2, 7.1 and 8.3

G)  $R_f$  value of 0.56 in the following chromatographic system:

a mixture of aqueous NaCl (87.5 g/l):aqueous  $\text{NaH}_2\text{PO}_4$  (0.5 g/l), and acetonitrile, 70:30, adjusted to pH 6.0

using reverse-phase silanized silica gel plates (RA-18 F<sub>254</sub>)

Visualization:

- U.V. light at 254 nm
- Yellow color with Pauly Reagent, i.e. diazotized sulfanilic acid (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))
- Bioautography using *B. subtilis* ATCC 6633 on minimal Davis medium.

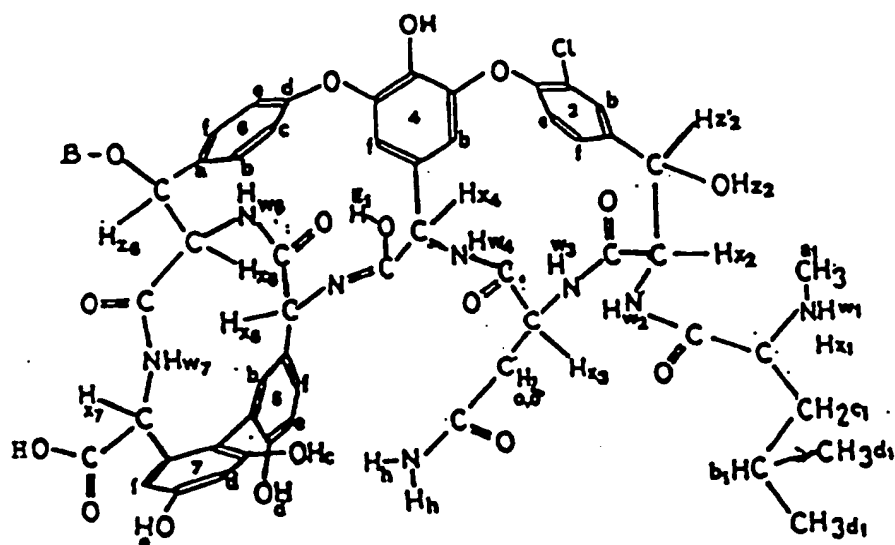
H) MW of about 1559 desumed from a FAB-MS spectrum showing the  $M + H^+$  peak at 1560.

4. A process according to claim 1, 2 or 3 wherein the strong acid is hydrochloric acid.
5. A process according to claim 1, 2, 3 or 4 wherein the solvent is dimethylsulfoxide or dimethylformamide.
6. A process according to claim 1, 2, 3, 4 or 5 wherein the reaction temperature is between 80 °C and 110 °C.
7. Use of a compound of claim 1 or 2 for preparing a medicament for use as an antibiotic.

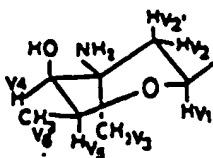
#### Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Antibiotikum A 42867 Pseudoaglycon und dessen pharmazeutisch verträgliche Salze mit der folgenden Formel (salzfreie Verbindung):



in der B eine beta-Vancosamin-Einheit der Formel bedeutet



Vancosamin  
( $\beta$ -Anomer)

und seine Tautomeren.

2. Antibiotikum A 42867 Pseudoaglycon und dessen pharmazeutisch verträgliche Salze mit den folgenden physikalisch-chemischen Parametern (salzfreie Verbindung):

A) Ultraviolett-Absorptionsspektrum, welches die folgenden Absorptions-Maxima zeigt:

	Lambda Max (nm)
a) 0.01 N HCl	279
b) Wasser	279 305 (Schulter)
c) Phosphat-Puffer pH-Wert 7,4	279
d) 0,1 N KOH	299 265 (Schulter)

B) Infrarot-Absorptionsspektrum in Nujol, welches die folgenden Absorptionsmaxima ( $\text{cm}^{-1}$ ) zeigt:

3700-3100; 3000-2800 (Nujol); 1650; 1590; 1460 (Nujol); 1375 (Nujol); 1305; 1240; 1210; 1160; 1130; 1060; 1010; 950; 870; 835; 720 (Nujol).

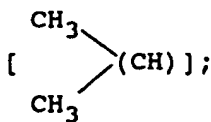
C)  $^1\text{H}$ -NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 250 MHz (Bruker Instruments) zeigt, aufgenommen in  $\text{DMSO-d}_6$  (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als interner Standard (0,00 ppm), ( $\Delta$  = ppm) :

Zucker-Einheit:

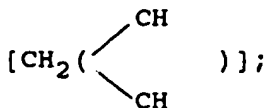
1,22, d [ $\text{CH}_3\text{-(CH)}$ ]; 1,50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ]; 2,25, m [ $\text{CH}_2\text{-(CH)}$ ]; 3,60, m [ $\text{CH-(CH}_3\text{)}$ ]; 4,90, d (anomeres Proton)

Rumpf-Einheit:

0,90, d und 0,94 d



1,74, m [ $\text{CH-(CH}_3\text{)}_2$ ];  
1,60, m



2,32, s [ $\text{CH}_3\text{-(NH)}$ ]; 2,49, s (Lösungsmittel  $\text{DMSO-d}_5$ ); 3,35, br [ $\text{H}_2\text{O}$ ]; 4,23-6,35 [aromatische und peptidische  $\text{CH}'\text{s}$ ]; 6,35-9,50 [aromatische  $\text{CH}'\text{s}$ , peptidische  $\text{NH}'\text{s}$  und phenolische  $\text{OH}'\text{s}$ ]

s = Singulett; d = Duplett; m = Multiplett; br = breit

D) Retentionszeit ( $R_t$ ) von 2,88 relativ zum Antibiotikum A 42867 ( $R_t = 8,45$  min), wenn durch Reversphasen-HPLC unter folgenden Bedingungen analysiert wurde:

Säule: Ultrasphere ODS (5 Mikrometer) Altex (Beckman) 4,6 mm (i.d.) x 250 mm

Vorsäule: 3 cm Brownlee Labs RP 18 (5 Mikrometer)

Eluentien:

A) (2,5 g/l)  $\text{NaH}_2\text{PO}_4/\text{CH}_3\text{CN}$ , 98:2 (v/v) auf einen pH-Wert von 6,0 eingestellt

B) (2,5 g/l)  $\text{NaH}_2\text{PO}_4/\text{CH}_3\text{CN}$ , 30:70 (v/v) auf einen pH-Wert von 6,0 eingestellt

Eluierungsart: linearer Gradient von 5% bis 60% von B in A in 40 min

Fließgeschwindigkeit: 1,8 ml/min

UV-Detektor: 254 nm

interner Standard: Antibiotikum A 42867 ( $R = 8,45$  min)

E) Elementar-Analyse zeigt die Gegenwart von einem Chloratom pro Molekül.

F) 2D  $^1\text{H-NMR-NOESY}$ -Analyse zeigt auf der Basis des Nuklear-Overhauser-Effekts (dipolare Kopplung durch den Raum) und skalarer Kopplung über chemische Bindungen, daß das Chloratom tatsächlich an Ring 2 und nicht an Ring 6 sitzt:

a) auf Grund skalarer Kopplung zeigt Proton 2b (siehe Formel I) aber keine ortho-Kopplung sondern nur eine meta-Kopplung mit Proton 2f; (Proton 2b sitzt am Ring 2 und nicht am Ring 6 auf Grund der folgenden dipolaren Kopplung:

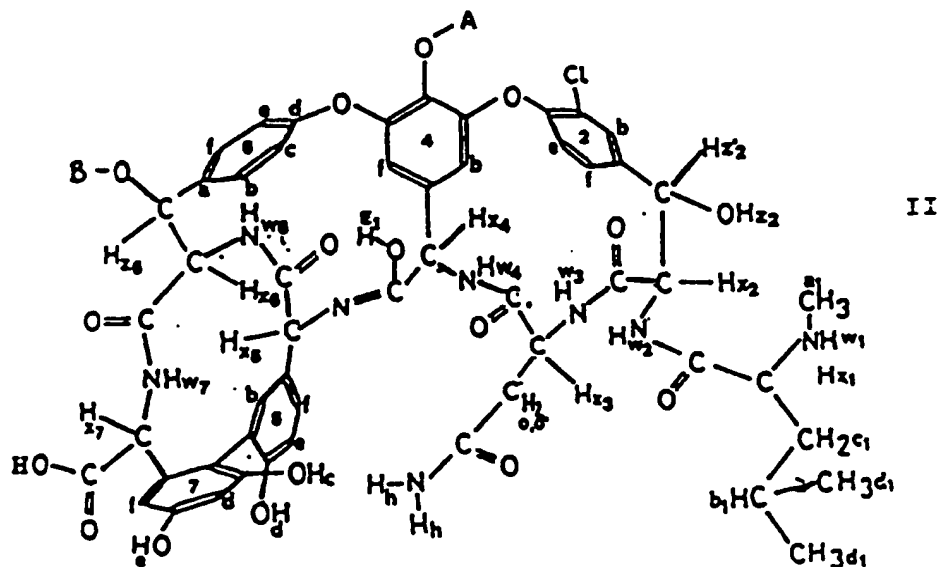
$2b \rightarrow z'_2$ ;  $2b \rightarrow (z_2)\text{OH}$ ;  $(z_2)\text{OH} \rightarrow z'_2$ ;  $z'_2 \rightarrow x_2$ )

b) auf Grund skalarer Kopplung zeigt Proton 6b (siehe Formel I) eine ortho-Kopplung mit Proton 6c (dipolare Kopplung  $6b \rightarrow 6c$ ;  $z_6 \rightarrow 6b$ ;  $z_6 \rightarrow x_6$ )

G) Ein Neutronen-Bombardierungs (FAB)-Massenspektrum mit  $\text{M} + \text{H}^+$  bei etwa 1251 (die theoretischen Clusterionen-Peaks für die Formel  $\text{C}_{60}\text{H}_{64}\text{N}_5\text{O}_{19}\text{Cl} + \text{H}$  liegen von etwa 1250 bis 1255, Durchschnittswert 1251,68). Das FAB-Spektrum wurde mit einem VG 70-250 Instrument mit Xenon als Bombardierungsgas erhalten; Strahlenergie 8 KeV; Beschleunigungsspannung 6 kV; Matrix Thioglycerol-Glycerol, 2:1.

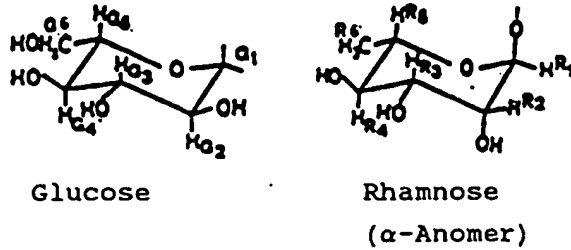
H) Säure- und Basenfunktionen sind fähig, Salze zu bilden.

3. Verfahren zur Herstellung einer Verbindung nach Anspruch 1 oder 2, umfassend die Umsetzung einer Verbindung der Formel II

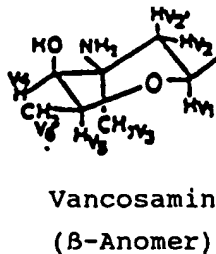


in der

A einen Disaccaridrest mit einer Einheit Glucose und einer Einheit d-Rhamnose, mit jeweils der Formel:



bedeutet, und  
B eine beta-Vancosamin-Einheit der Formel bedeutet:



oder eines Tautomers davon,  
unter kontrollierter Säure-Hydrolyse in einem organischen Lösungsmittel und in Gegenwart einer starken Säure.

4. Verfahren zur Herstellung einer Verbindung nach Anspruch 1 oder 2, umfassend die Umsetzung von Antibiotikum A 42867, welches die folgenden Merkmale hat:

A) Ultraviolett-Absorptionsspektrum, welches die folgenden Absorptions-Maxima zeigt:

	Lambda Max (nm)
a) 0,1 N HCl	282
b) Wasser	282
c) Phosphat-Puffer pH-Wert 7,4	282
d) Phosphat-Puffer pH-Wert 9	282 305 (Schulter)
e) 0,1 N KOH	305 265 (Schulter)

B) Infrarot-Absorptionsspektrum, welches die folgenden Absorptionsmaxima ( $\text{cm}^{-1}$ ) zeigt:

3700-3100; 3000-2800 (Nujol); 1650; 1580; 1460 (Nujol); 1375 (Nujol); 1300; 1235; 1210; 1160; 1130; 1060; 1025; 1000; 970; 840; 790-700; 720 (Nujol).

C)  $^1\text{H}$ -NMR Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 500 MHz zeigt, aufgenommen in  $\text{DMSO}-d_6$  (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als interner Standard (0,00 ppm), ( $\Delta$  = ppm) :

$d_1$  0,90;  $R_6$  1,06;  $V_6$  1,23;  $V_3$  1,52;  $c_1$  1,62;  $b_1$  1,76;  $V_2, V_2'$  2,30;  $a_1$  (N-CH<sub>3</sub>) 2,38;  $R_4$  3,12;  $x_1, R_3, V_4$  3,10-3,50;  $V_5$  3,60;  $R_2$  3,79;  $x_6, R_5$  4,22;  $x_7$  4,51;  $x_5$  4,75;  $V_1$  4,88;  $R_1$  4,96;  $x_2$  5,02;  $x_3, z_6$  5,12;  $z'_2$  5,22;  $4f$  5,33;  $4b$  5,53;  $z_2$  (OH) 5,88;  $7f$  6,23;  $x_4$  6,34;  $7d$  6,41;  $h$  6,62;  $5c, w_3$  6,76;  $5f$  6,84;  $6c$  7,12;  $5b$  7,15;  $2b$  7,26;  $w_6, h$  7,32;  $6b$  7,50;  $w_4$  8,15;  $w_7$  8,45;  $7c$  9,10;  $5d, e_1$  9,32;  $7e$  9,39;



D) Retentionszeit ( $R_t$ ) von 0,665 relativ zum Vancomycin.HCl (Vancocin, Eli Lilly,  $R_t$  = 9,96 min), wenn durch Reversphasen-HPLC unter folgenden Bedingungen analysiert wurde:

Säule: Ultrasphere ODS (5 Mikrometer) Altex (Beckman) 4,6 mm (i.d.) x 250 mm

Vorsäule: Brownlee Labs RP 18 (5 Mikrometer)

Eluent A:

CH<sub>3</sub>CN 2% } eingestellt  
(2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 98% } auf pH 6,0

Eluent B:

CH<sub>3</sub>CN 70% } eingestellt  
(2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 30% } auf pH 6,0

Eluierungsart: linearer Gradient von 5% bis 60% von Eluent B in Eluent A in 40 min

Fließgeschwindigkeit: 1,6 ml/min

UV-Detektor: 254 nm

interner Standard: Vancomycin.HCl ( $R_t$  = 9,96 min) (Vancocin, Eli Lilly)

E) Elementar-Analyse, nachdem die Probe vorher bei etwa 140 °C unter inerter Atmosphäre getrocknet wurde, welche die folgenden Circa-Prozent-Zusammensetzungen (Durchschnitt) angibt: Kohlenstoff 53,3%; Wasserstoff 5,9%; Stickstoff 7,85%; Chlor (gesamt) 4,41%; Chlor (ionisch) 2,22%. Anorganischer Rest bei 900 °C in der Luft: 0,875%.

F) Säure-Base-Titrationsprofil in Wasser bei einer Titration mit 0,05 N wäßriger KOH einer Probe, zu welcher vorher ein Überschuß an wäßriger HCl gegeben wurde, zeigt pKa-Werte bei 3,2, 7,1 und 8,3

G)  $R_f$ -Wert von 0,56 im folgenden Chromatographie-System

ein Gemisch von wäßriger NaCl (87,5 g/l): wäßrige NaH<sub>2</sub>PO<sub>4</sub> (0,5 g/l) und Acetonitril, 70:30, eingestellt auf einen pH-Wert von 6,0

unter Verwendung von Reversphasen-silanisiertem Silicagel-Platten (RA-18 F<sub>254</sub>)

Sichtbarmachen:

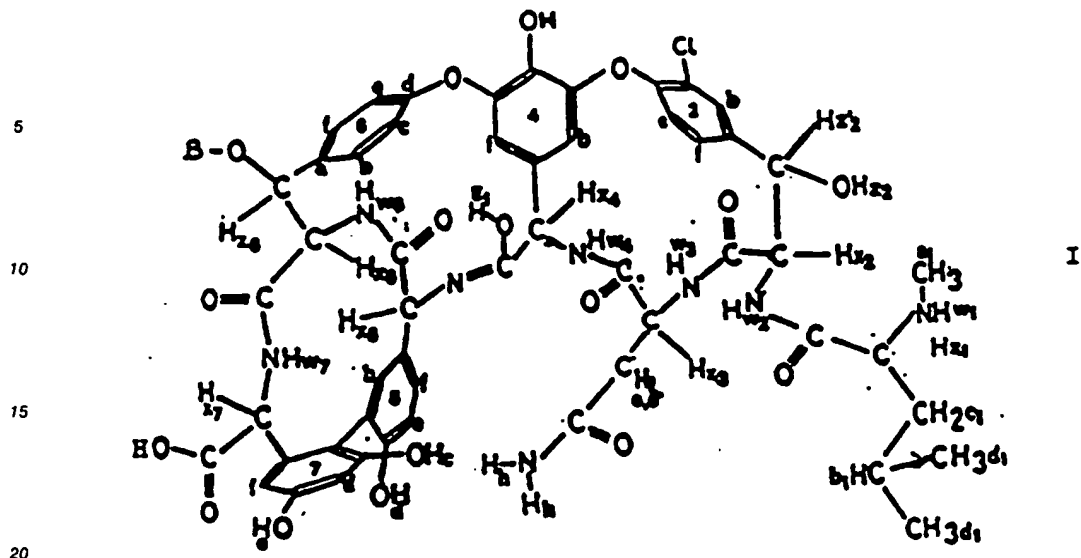
- UV-Licht bei 254 nm
- gelbe Farbe mit Pauly Reagenz, d.h. diazotierte Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))
- Bioautographie unter Verwendung von *B. subtilis* ATCC 6633 auf minimalem Davis-Medium.

h) MW von etwa 1559, erhalten aus einem FAB-MS-Spektrum mit dem M + H<sup>+</sup>-Peak bei 1560.

5. Verfahren gemäß Anspruch 3, wobei die starke Säure Salzsäure ist.
6. Verfahren gemäß Anspruch 3, wobei das Lösungsmittel Dimethylsulfoxid oder Dimethylformamid ist.
7. Verfahren gemäß Anspruch 3, wobei die Reaktionstemperatur zwischen 80 °C und 110 °C liegt.
8. Verwendung einer Verbindung nach Anspruch 1 oder 2 zur Herstellung eines Medikaments zur Verwendung als Antibiotikum.
9. Arzneimittel, umfassend eine Verbindung nach Anspruch 1 im Gemisch mit einem pharmazeutisch verträglichen Träger.
10. Verbindung nach Anspruch 1 oder 2 zur Verwendung als Medikament.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Ein Verfahren zur Herstellung von Antibiotikum A 42867 Pseudoaglycon und dessen pharmazeutisch verträglichen Salzen mit der folgenden Formel (salzfreie Verbindung):



in der  
B eine beta-Vancosamin-Einheit der Formel bedeutet:



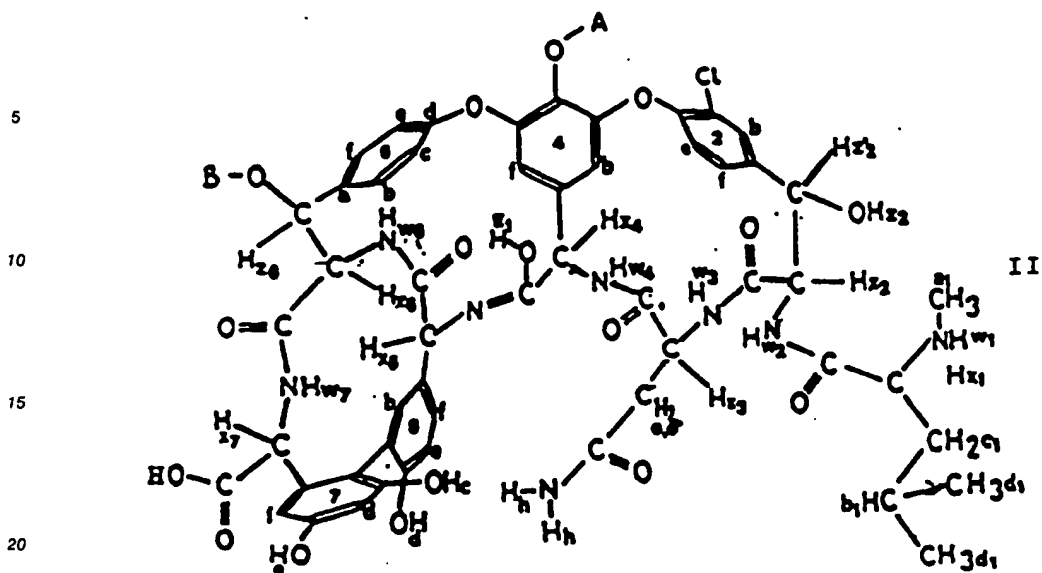
und seiner Tautomeren, umfassend die Umsetzung einer Verbindung der Formel II

40

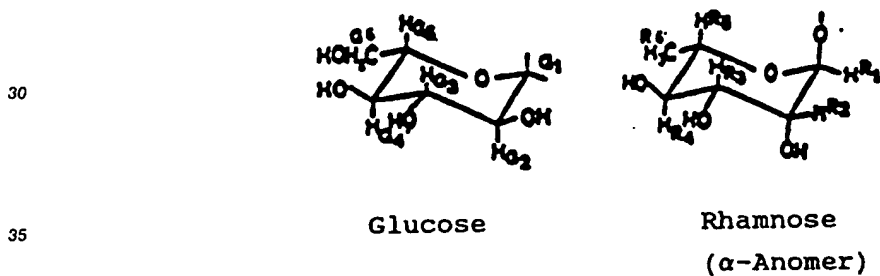
45

50

55



in der A einen Disaccharid-Rest mit einer Einheit Glucose und einer Einheit d-Rhamnose der  
25 jeweiligen Formel



und  
40 B eine beta-Vancosamin-Einheit der Formel bedeutet:



55 oder eines Tautomers davon,  
unter kontrollierter Säure-Hydrolyse in einem organischen Lösungsmittel und in Gegenwart einer  
starken Säure.

2. Verfahren zur Herstellung von Antibiotikum A 42867 Pseudoaglycon und dessen pharmazeutisch verträgliche Salze mit den folgenden physikalisch-chemischen Parametern (salzfreie Verbindung):

A) Ultraviolett-Absorptionsspektrum, welches die folgenden Absorptions-Maxima zeigt:

5

	Lambda Max (nm)
a) 0.01 N HCl	279
b) Wasser	279 305 (Schulter)
c) Phosphat-Puffer pH-Wert 7,4	279
d) 0,1 N KOH	299 265 (Schulter)

10

15

B) Infrarot-Absorptionsspektrum in Nujol, welches die folgenden Absorptionsmaxima ( $\text{cm}^{-1}$ ) zeigt:

3700-3100, 3000-2800 (Nujol); 1650; 1590; 1460 (Nujol); 1375 (Nujol); 1305; 1240; 1210; 1160; 1130; 1060; 1010; 950; 870; 835; 720 (Nujol).

20

C)  $^1\text{H}$ -NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 250 MHz (Bruker Instruments) zeigt, aufgenommen in  $\text{DMSO-d}_6$  (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als interner Standard (0,00 ppm), (Delta = ppm) :

Zucker-Einheit:

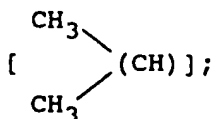
1,22, d [ $\text{CH}_3\text{-(CH)}$ ]; 1,50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ]; 2,25, m [ $\text{CH}_2\text{-(CH)}$ ]; 3,60, m [ $\text{CH-(CH}_3\text{)}$ ]; 4,90, d (anomerer Proton)

25

Rumpf-Einheit:

0,90, d und 0,94 d

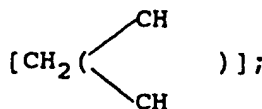
30



35

1,74, m [ $\text{CH-(CH}_3\text{)}_2$ ];  
1,60, m

40



45

2,32, s [ $\text{CH}_3\text{-(NH)}$ ]; 2,49, s (Lösungsmittel  $\text{DMSO-d}_6$ ); 3,35, br [ $\text{H}_2\text{O}$ ]; 4,23-6,35 [aromatische und peptidische CH's]; 6,35-9,50 [aromatische CH's, peptidische NH's und phenolische OH's]

s = Singulett; d = Duplett; m = Multiplett; br = breit

D) Retentionszeit ( $R_t$ ) von 2,88 relativ zum Antibiotikum A 42867 ( $R_t$  = 8,45 min), wenn durch Reversphasen-HPLC unter folgenden Bedingungen analysiert wurde:

Säule: Ultrasphere ODS (5 Mikrometer) Altex (Beckman) 4,6 mm (i.d.) x 250 mm

50

Vorsäule: 3 cm Brownlee Labs RP 18 (5 Mikrometer)

Eluentien:

A) (2,5 g/l)  $\text{NaH}_2\text{PO}_4/\text{CH}_3\text{CN}$ , 98:2 (v/v) auf einen pH-Wert von 6,0 eingestellt

B) (2,5 g/l)  $\text{NaH}_2\text{PO}_4/\text{CH}_3\text{CN}$ , 30:70 (v/v) auf einen pH-Wert von 6,0 eingestellt

Eluierungsart: linearer Gradient von 5% bis 60% von B in A in 40 min

55

Fließgeschwindigkeit: 1,8 ml/min

UV-Detektor: 254 nm

interner Standard: Antibiotikum A 42867 ( $R$  = 8,45 min)

E)Elementar-Analyse zeigt die Gegenwart von einem Chloratom pro Molekül.

F) 2D <sup>1</sup>H-NMR-NOESY-Analysis zeigt auf der Basis des Nuklear-Overhauser-Effekts (dipolare Kopplung durch den Raum) und skalarer Kopplung über chemische Bindungen, daß das Chloratom tatsächlich an Ring 2 und nicht an Ring 6 sitzt:

a) auf Grund skalarer Kopplung zeigt Proton 2b (siehe Formel I) keine ortho-Kopplung, sondern nur eine meta-Kopplung mit Proton 2f; (Proton 2b sitzt am Ring 2 und nicht am Ring 6 auf Grund der folgenden dipolaren Kopplung:

$2b \rightarrow z'_2$ ;  $2b \rightarrow (z_2)OH$ ;  $(z_2)OH \rightarrow z'_2$ ;  $z'_2 \rightarrow x_2$

b) auf Grund skalarer Kopplung zeigt Proton 6b (siehe Formel I) eine ortho-Kopplung mit Proton 6c (dipolare Kopplung  $6b \rightarrow 6c$ ;  $z_6 \rightarrow 6b$ ;  $z_6 \rightarrow x_6$ )

G) Ein Neutronen-Bombardierungs (FAB)-Massenspektrum mit  $M + H^+$  bei etwa 1251 (die theoretischen Clusterionen-Peaks für die Formel  $C_{60}H_6N_9O_{19}Cl + H$  liegen von etwa 1250 bis 1255, Durchschnittswert 1251,68). Das FAB-Spektrum wurde mit einem VG 70-250 Instrument mit Xenon als Bombardierungsgas erhalten; Strahlenergie 8 KeV; Beschleunigungsspannung 6 kV; Matrix Thioglycerol-Glycerol, 2:1.

H) Säure- und Basenfunktionen sind fähig, Salze zu bilden.

3. Verfahren zur Herstellung einer Verbindung nach Anspruch 1 oder 2, umfassend die Umsetzung von Antibiotikum A 42867 mit folgenden Merkmalen:

A) Ultraviolet-Absorptionsspektrum, welches die folgenden Absorptions-Maxima zeigt:

	Lambda Max (nm)
a) 0,1 N HCl	282
b) Wasser	282
c) Phosphat-Puffer pH-Wert 7,4	282
d) Phosphat-Puffer pH-Wert 9	282 305 (Schulter)
e) 0,1 N KOH	305 265 (Schulter)

B) Infrarot-Absorptionsspektrum, welches die folgenden Absorptionsmaxima ( $cm^{-1}$ ) zeigt:

3700-3100; 3000-2800 (Nujol); 1650; 1580; 1460 (Nujol); 1375 (Nujol); 1300; 1235; 1210; 1160; 1130; 1060; 1025; 1000; 970; 840; 790-700; 720 (Nujol).

C) <sup>1</sup>H-NMR-Spektrum, welches die folgenden Gruppen von Signalen (in ppm) bei 500 MHz zeigt, aufgenommen in DMSO- $d_6$  (Hexadeuterodimethylsulfoxid) unter Verwendung von TMS als interner Standard (0,00 ppm), ( $\Delta$  = ppm):

$d_1$  0,90;  $R_6$  1,06;  $V_6$  1,23;  $V_3$  1,52;  $c_1$  1,62;  $b_1$  1,76;  $V_2, V_2'$  2,30;  $a_1$  (N-CH<sub>3</sub>) 2,38;  $R_4$  3,12;  $x_1, R_3, V_4$  3,10-3,50;  $V_5$  3,60;  $R_2$  3,79;  $x_6, R_5$  4,22;  $x_7$  4,51;  $x_5$  4,75;  $V_1$  4,88;  $R_1$  4,96;  $x_2$  5,02;  $x_3, z_6$  5,12;  $z'_2$  5,22;  $4f$  5,33;  $4b$  5,53;  $z_2(OH)$  5,88;  $7f$  6,23;  $x_4$  6,34;  $7d$  6,41;  $h$  6,62;  $5c, w_3$  6,76;  $5f$  6,84;  $6c$  7,12;  $5b$  7,15;  $2b$  7,26;  $w_6, h$  7,32;  $6b$  7,50;  $w_4$  8,15;  $w_7$  8,45;  $7c$  9,10;  $5d, e_1$  9,32;  $7e$  9,39;

D) Retentionszeit ( $R_t$ ) von 0,665 relativ zum Vancomycin.HCl (Vancocin, Eli Lilly,  $R_t$  = 9,96 min), wenn durch Reversphasen-HPLC unter folgenden Bedingungen analysiert wurde:

Säule: Ultrasphere ODS (5 Mikrometer) Altex (Beckman) 4,6 mm (i.d.) x 250 mm

Vorsäule: Brownlee Labs RP 18 (5 Mikrometer)

Eluent A:

$CH_3CN$  2% } eingestellt  
(2,5 g/l)  $NaH_2PO_4 \cdot H_2O$  98% } auf pH 6,0

Eluent B:

$CH_3CN$  70% } eingestellt  
(2,5 g/l)  $NaH_2PO_4 \cdot H_2O$  30% } auf pH 6,0

Eluierungsart: linearer Gradient von 5% bis 60% von Eluent B in Eluent A in 40 min

Fließgeschwindigkeit: 1,6 ml/min

UV-Detektor: 254 nm

interner Standard: Vancomycin.HCl ( $R_f = 9,96$  min) (Vancocin, Eli Lilly)

E) Elementar-Analyse, nachdem die Probe vorher bei etwa  $140^\circ\text{C}$  unter inerter Atmosphäre getrocknet wurde, welche die folgenden Circa-Prozent-Zusammensetzungen (Durchschnitt) angibt: Kohlenstoff 53,3%; Wasserstoff 5,9%; Stickstoff 7,85%; Chlor (gesamt) 4,41%; Chlor (ionisch) 2,22%. Anorganischer Rest bei  $900^\circ\text{C}$  in der Luft: 0,875%.

F) Säure-Base-Titrationsprofil in Wasser bei einer Titration mit 0,05 N wäßriger KOH einer Probe, zu welcher vorher ein Überschuß an wäßriger HCl gegeben wurde, zeigt pKa-Werte bei 3,2, 7,1 und 8,3

G)  $R_f$ -Wert von 0,56 im folgenden Chromatographie-System

ein Gemisch von wäßriger NaCl (87,5 g/l): wäßrige  $\text{NaH}_2\text{PO}_4$  (0,5 g/l) und Acetonitril, 70:30, eingestellt auf einen pH-Wert von 6,0

unter Verwendung von Reversphasen-silanisiertem Silicagel-Platten (RA-18 F<sub>254</sub>)

Sichtbarmachen:

- UV-Licht bei 254 nm
- gelbe Farbe mit Pauly Reagenz, d.h. diazierte Sulfanilsäure (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))
- Bioautographie unter Verwendung von *B. subtilis* ATCC 6633 auf minimalem Davis-Medium.

H) MW von etwa 1559, erhalten aus einem FAB-MS-Spektrum mit dem  $\text{M} + \text{H}^+$ -Peak bei 1560.

4. Verfahren gemäß Anspruch 1, 2, oder 3, wobei die starke Säure Salzsäure ist.

5. Verfahren gemäß Anspruch 1, 2, 3 oder 4, wobei das Lösungsmittel Dimethylsulfoxid oder Dimethylformamid ist.

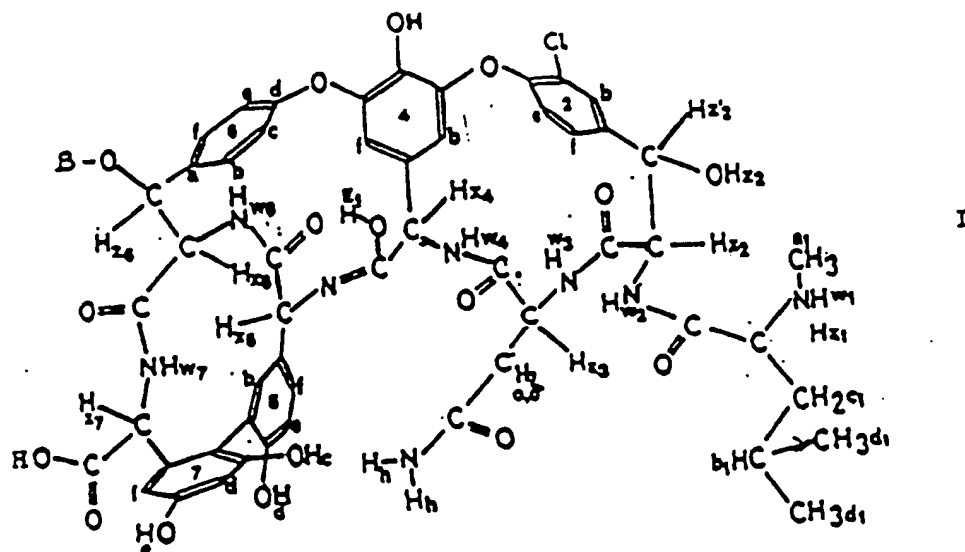
6. Verfahren gemäß Anspruch 1, 2, 3, 4 oder 5, wobei die Reaktionstemperatur zwischen  $80^\circ\text{C}$  und  $110^\circ\text{C}$  liegt.

7. Verwendung einer Verbindung nach Anspruch 1 oder 2 zur Herstellung eines Medikaments zur Verwendung als Antibiotikum.

#### Revendications

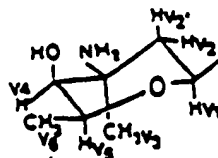
Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Antibiotique A 42867 pseudoaglycone et ses sels pharmaceutiquement acceptables, ayant la formule suivante (composé non salifié) :



dans laquelle

B représente un motif bêta-vancosamine de formule



VANCOSAMINE  
(anomère  $\beta$ )

et ses tautomères.

2. Antibiotique A 42867 pseudoaglycone et ses sels pharmaceutiquement acceptables, ayant les paramètres physico-chimiques suivants (forme non salifiée) :

A) Spectre d'absorption UV qui présente les maxima d'absorption suivants :

	Lambda max (nm)
a) HCl 0,1N	279
b) Eau	279 305 (épaulement)
c) Tampon phosphate pH 7,4	279
d) KOH 0,1N	299 265 (épaulement)

B) Spectre d'absorption infra-rouge dans une pâte de nujol qui présente les maxima d'absorption suivants ( $\text{cm}^{-1}$ ) :

3700-3100, 3000-2800 (nujol) ; 1650 ; 1590 ; 1460 (nujol) ; 1375 (nujol) ; 1305 ; 1240 ; 1210 ; 1160 ; 1130 ; 1060 ; 1010 ; 950 ; 870 ; 835 ; 720 (nujol)

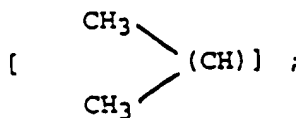
C) Spectre RMN<sup>1</sup>H qui présente les groupes suivants de signaux (en ppm) à 250 MHz (Bruker Instruments) enregistré dans du DMSO-d<sub>6</sub> (hexadeutérodiméthylsulfoxyde) à l'aide de TMS en tant que référence interne (0,00 ppm), ( $\delta$  = ppm) :

Fragment sucre :

1,22, d [ $\text{CH}_2\text{-(CH)}$ ] ; 1,50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ] ; 2,25, m [ $\text{CH}_2\text{-(CH)}$ ] ; 3,60, m [ $\text{CH-(CH}_3\text{)}$ ] ; 4,90, d (proton anomère)

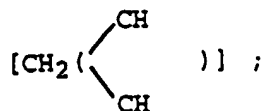
Fragment noyau :

0,90, d et 0,94, d



1,74, m [ $\text{CH(CH}_3\text{)}_2$ ] ;

1,60, m



5

2,32, s [CH<sub>3</sub>-(NH)] ; 2,49, s (solvant DMSO-d<sub>5</sub>) ; 3,35, t [H<sub>2</sub>O] ; 4,23-6,35 [CH aromatiques et peptidiques] ; 6,35-9,50 [CH aromatiques, NH peptidiques et OH phénoliques]

s = singulet, d = doublet, m = multiplet, t = large

10 D) Temps de rétention (R<sub>t</sub>) égal à 2,88 par rapport à l'antibiotique A 42867 (R<sub>t</sub> = 8,45 mn), l'analyse étant effectuée par CLHP en phase inversée dans les conditions suivantes :

colonne : Ultrasphère ODS (5 micromètres) Altex (Beckman), diamètre intérieur 4,6 mm x 250 mm.

pré-colonne : Brownlee Labs RP 18, 3 cm (5 micromètres)

15 éluants :

A) (2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN, 98:2 (v/v), ajusté à pH 6,0

B) (2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN, 30:70 (v/v), ajusté à pH 6,0

Mode d'élution : gradient linéaire de 5 à 60 % de B dans A en 40 mn

débit : 1,8 ml/mn

20 détecteur UV : 254 nm

étalon interne : antibiotique A 42867 (R<sub>t</sub> = 8,45 mn)

E) Analyse élémentaire montrant la présence d'un atome de chlore par molécule

F) Analyse NOESYPH RMN-<sup>1</sup>H 2D, montrant que l'atome de chlore moléculaire se trouve sur le cycle 2 et non sur le cycle 6, sur la base de l'effet Nucléaire Overhauser (couplage dipolaire dans l'espace) et du couplage scalaire sur la liaison chimique, et plus précisément :

25 a) sur la base du couplage scalaire, le proton 2b (voir formule I) ne montre qu'un couplage méta avec le proton 2f mais pas de couplage ortho ; (le proton 2b se trouve sur le cycle 2 et non sur le cycle 6, sur la base du couplage dipolaire suivant :

2b → z'<sub>2</sub> ; 2b → (z<sub>2</sub>)OH ; (z<sub>2</sub>)OH → z'<sub>2</sub> ; z'<sub>2</sub> → x<sub>2</sub>)

30 b) sur la base du couplage scalaire, le proton 6b (voir formule I) montre un couplage ortho avec le proton 6c (couplage dipolaire 6b → 6c ; z<sub>6</sub> → 6b ; z<sub>6</sub> → x<sub>6</sub>).

G) Un spectre de masse par bombardement atomique rapide (FAB), avec M + H<sup>+</sup> à environ 1251 (les pics ioniques des agrégats théoriques, pour la formule C<sub>60</sub>H<sub>64</sub>N<sub>9</sub>O<sub>15</sub>Cl + H, vont de 1250 à 1255, valeur moyenne 1251,68). Le spectre FAB est obtenu avec un appareil VG 70-250, le gaz de bombardement étant le Xénon ; énergie du faisceau : 8 keV ; tension d'accélération 6 kV ; matrice thioglycérol-glycérol, 2:1.

35 H) Fonctions acides et basiques pouvant former des sels.

3. Procédé pour préparer un composé selon la revendication 1 ou 2, qui comprend le fait de faire réagir  
40 un composé de formule II

45

50

55





25

dans laquelle

A représente un résidu disaccharidique d'un motif de glucose et d'un motif de d-rhamnose, ayant respectivement les formules suivantes :



## GLUCOSE



(anomère  $\alpha$ )

40

et

B représente un motif bêta-vancosamine de formule



VANCOSAMINE

(anomère  $\beta$ )

55

ou l'un de ses tautomères,

sous hydrolyse acide contrôlée dans un solvant organique et en présence d'un acide fort.

4. Procédé pour préparer un composé selon la revendication 1 ou 2, qui comprend le fait de faire réagir un antibiotique A 42867, ayant les caractéristiques suivantes :

A) Spectre d'absorption UV, qui présente les maxima d'absorption suivants :

	Lambda max (nm)
a) HCl 0,1N	282
b) eau	282
c) tampon phosphate pH 7,4	282
d) tampon phosphate pH 9	282 305 (épaulement)
e) KOH 0,1N	305 265 (épaulement)

B) Spectre d'absorption infra-rouge, qui présente les maxima d'absorption suivants ( $\text{cm}^{-1}$ ) :

3700-3100, 3000-2800 (nujol) ; 1650 ; 1580 ; 1460 (nujol) ; 1375 (nujol) ; 1300 ; 1235 ; 1210 ; 1160 ; 1130 ; 1060 ; 1025 ; 1000 ; 970 ; 840 ; 790-700 ; 720 (nujol)

C) Spectre RMN<sup>1</sup>H, qui présente les groupes de signaux suivants (en ppm) à 500 MHz, enregistré dans du DMSO- $\text{d}_6$  (hexadéutérodiméthylsulfoxyde), la référence interne étant le TMS (0,00 ppm), ( $\delta$  = ppm) :

$\text{d}_1$  0,90 ;  $\text{R}_6$  1,06 ;  $\text{V}_6$  1,23 ;  $\text{V}_3$  1,52 ;  $\text{c}_1$  1,62 ;  $\text{b}_1$  1,76 ;  $\text{V}_2, \text{V}_2'$  2,30 ;  $\text{a}_1$  (N- $\text{CH}_3$ ) 2,38 ;  $\text{R}_4$  3,12 ;  $\text{x}_1, \text{R}_3, \text{V}_4$  3,10-3,50 ;  $\text{V}_5$  3,60 ;  $\text{R}_2$  3,79 ;  $\text{x}_6, \text{R}_5$  4,22 ;  $\text{x}_7$  4,51 ;  $\text{x}_5$  4,75 ;  $\text{V}_1$  4,88 ;  $\text{R}_1$  4,96 ;  $\text{x}_2$  5,02 ;  $\text{x}_3, \text{z}_6$  5,12 ;  $\text{z}'_2$  5,22 ;  $4\text{f}$  5,33 ;  $4\text{b}$  5,53 ;  $\text{z}_2$  (OH) 5,88 ;  $7\text{f}$  6,23 ;  $\text{x}_4$  6,34 ;  $7\text{d}$  6,41 ;  $\text{h}$  6,62 ;  $5\text{c}, \text{w}_3$  6,76 ;  $5\text{f}$  6,84 ;  $6\text{c}$  7,12 ;  $5\text{b}$  7,15 ;  $2\text{b}$  7,26 ;  $\text{w}_6, \text{h}$  7,32 ;  $6\text{b}$  7,50 ;  $\text{w}_4$  8,15 ;  $\text{w}_7$  8,45 ;  $7\text{c}$  9,10 ;  $5\text{d}, \text{e}_1$  9,32 ;  $7\text{e}$  9,39 ;

D) Temps de rétention ( $\text{R}_t$ ) égal à 0,665 par rapport à la Vancomycine.HCl (Vancocin, Eli Lilly,  $\text{R}_t$  = 9,96 mn), l'analyse étant effectuée par CLHP en phase inversée dans les conditions suivantes :

colonne : Ultrasphère ODS (5 micromètres) Altex (Beckman), diamètre intérieur 4,6 mm x 250 mm

pré-colonne : Brownlee Labs RP 18 (5 micromètres)

éluant A :

$\text{CH}_3\text{CN}$  : 2 %  
(2,5 g/l)  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  : 98 % } ajusté à pH 6,0

éluant B :

$\text{CH}_3\text{CN}$  : 70 %  
(2,5 g/l)  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  : 30 % } ajusté à pH 6,0

élution : gradient linéaire de 5 à 60 % de l'éluant B dans l'éluant A, en 40 mn

débit : 1,6 ml/mn

détecteur UV : 254 nm

étalon interne : Vancomycine.HCl ( $\text{R}_t$  = 9,96 mn) (Vancocin, Eli Lilly)

E) Analyse élémentaire, après séchage préalable de l'échantillon à environ 140 °C sous atmosphère inerte, qui indique la composition approximative suivante en pourcentage (moyenne) : carbone 53,3 % ; hydrogène 5,9 % ; azote 7,85 % ; chlore (total) 4,41 % ; chlore (ionique) 2,22 %. Résidu minéral à 900 °C dans l'air : 0,875 %.

F) Profil de titrage acide-base dans l'eau après titrage avec une solution aqueuse 0,05N de KOH, effectué sur un échantillon préalablement additionné d'un excès de HCl en solution aqueuse, qui présente des valeurs de pKa de 3,2, 7,1 et 8,3.

G)  $R_1$  de 0,56 dans le système chromatographique suivant :  
un mélange 70:30, ajusté à pH 6,0, de NaCl en solution aqueuse (87,5 g/l)/ $\text{NaH}_2\text{PO}_4$  en solution aqueuse (0,5 g/l) et d'acétonitrile

utilisation de plaques de gel de silice silanisé en phase inversée (RA-18 F<sub>254</sub>).

Visualisation :

- lumière UV à 254 nm
- couleur jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))
- bio-autographie avec *B. subtilis* ATCC 6633 sur un milieu minimal de Davis.

H) Poids moléculaire d'environ 1559, d'après un spectre de masse par bombardement atomique rapide (FAB-MS) présentant le pic  $M + H^+$  à 1560.

5. Procédé selon la revendication 3, dans lequel l'acide fort est l'acide chlorhydrique.

6. Procédé selon la revendication 3, dans lequel le solvant est le diméthylsulfoxyde ou le diméthylformamide.

7. Procédé selon la revendication 3, dans lequel la température de réaction est comprise entre 80 et 110°C.

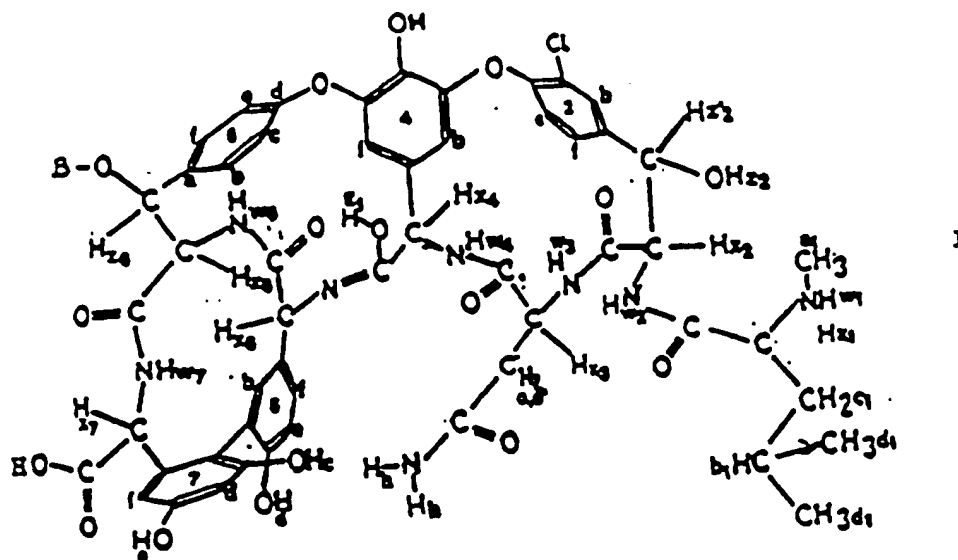
8. Utilisation d'un composé selon la revendication 1 ou 2 pour préparer un médicament destiné à être utilisé comme antibiotique.

9. Composition pharmaceutique comprenant un composé selon la revendication 1 en mélange avec un véhicule pharmaceutiquement acceptable.

10. Composé selon la revendication 1 ou 2, pour utilisation en tant que médicament.

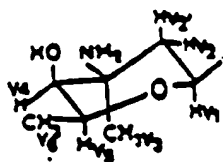
Revendications pour les Etats contractants suivants : ES, GR

1. Procédé pour préparer l'antibiotique A 42867 pseudoaglycone et ses sels pharmaceutiquement acceptables, ayant la formule suivante (composé non salifié) :



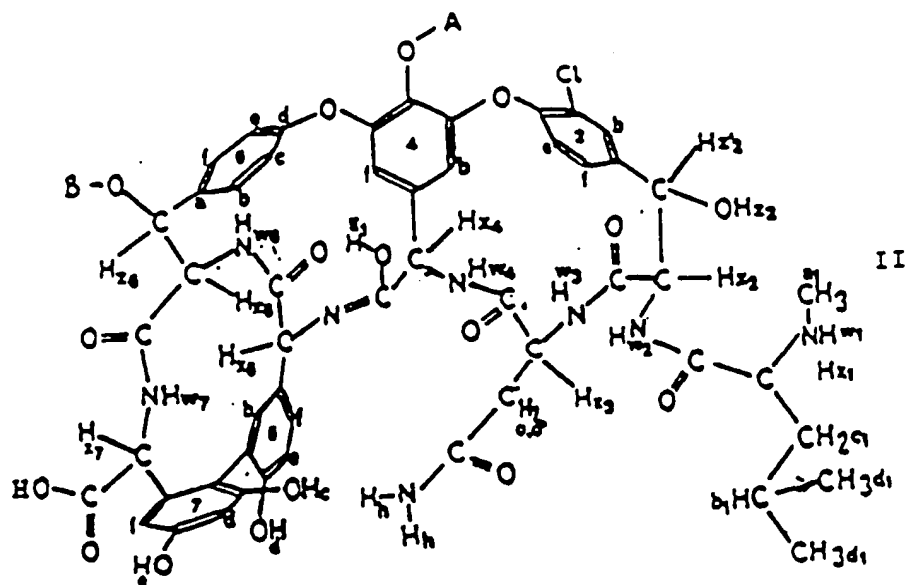
dans laquelle

B représente un motif bêta-vancosamine de formule



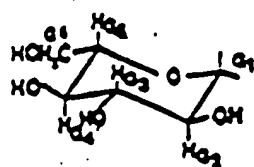
VANCOSAMINE  
(anomère  $\beta$ )

et ses tautomères, qui comprend le fait de faire réagir un composé de formule II

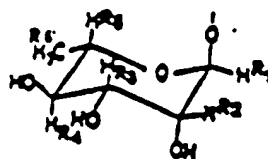


dans laquelle

A représente un résidu disaccharidique d'un motif de glucose et d'un motif de d-rhamnose, ayant respectivement les formules suivantes :



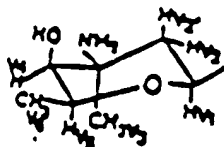
GLUCOSE



RHAMNOSE  
(anomère  $\alpha$ )

et

B représente un motif bêta-vancosamine de formule



VANCOSAMINE  
(anomère  $\beta$ )

ou l'un de ses tautomères,  
sous hydrolyse acide contrôlée dans un solvant organique et en présence d'un acide fort.

2. Procédé pour préparer l'antibiotique A 42867 pseudoaglycone et ses sels pharmaceutiquement acceptables, ayant les paramètres physico-chimiques suivants (forme non salifiée) :

A) Spectre d'absorption UV qui présente les maxima d'absorption suivants :

	Lambda max (nm)
a) HCl 0,1N	279
b) Eau	279 305 (épaulement)
c) Tampon phosphate pH 7,4	279
d) KOH 0,1N	299 265 (épaulement)

B) Spectre d'absorption infra-rouge dans une pâte de nujol qui présente les maxima d'absorption suivants ( $\text{cm}^{-1}$ ) :

3700-3100, 3000-2800 (nujol) ; 1650 ; 1590 ; 1460 (nujol) ; 1375 (nujol) ; 1305 ; 1240 ; 1210 ; 1160 ; 1130 ; 1060 ; 1010 ; 950 ; 870 ; 835 ; 720 (nujol)

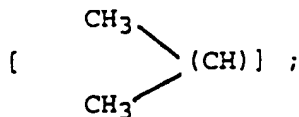
C) Spectre RMN<sup>1</sup>H qui présente les groupes suivants de signaux (en ppm) à 250 MHz (Bruker Instruments) enregistré dans du DMSO- $\text{d}_6$  (hexadéutériodiméthylsulfoxyde) à l'aide de TMS en tant que référence interne (0,00 ppm), ( $\delta$  = ppm) :

Fragment sucre :

1,22, d [ $\text{CH}_3\text{-(CH)}$ ] ; 1,50, s [ $\text{CH}_3\text{-C(NH}_2\text{)}$ ] ; 2,25, m [ $\text{CH}_2\text{-(CH)}$ ] ; 3,60, m [ $\text{CH-(CH}_3\text{)}$ ] ; 4,90, d (proton anomère)

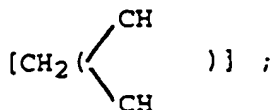
Fragment noyau :

0,90, d et 0,94, d



1,74, m [ $\text{CH(CH}_3\text{)}_2$ ] ;

1,60, m



# EP 0 326 029 B1

2,32, s [CH<sub>3</sub>-(NH)] ; 2,49, s (solvant DMSO-d<sub>5</sub>) ; 3,35, t [H<sub>2</sub>O] ; 4,23-6,35 [CH aromatiques et peptidiques] ; 6,35-9,50 [CH aromatiques, NH peptidiques et OH phénoliques]

s = singulet, d = doublet, m = multiplet, l = large

D) Temps de rétention (R<sub>t</sub>) égal à 2,88 par rapport à l'antibiotique A 42867 (R<sub>t</sub> = 8,45 mn), l'analyse étant effectuée par CLHP en phase inversée dans les conditions suivantes :

colonne : Ultrasphère ODS (5 micromètres) Altex (Beckman), diamètre intérieur 4,6 mm x 250 mm.

pré-colonne : Brownlee Labs RP 18, 3 cm (5 micromètres)

éluants :

A) (2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN, 98:2 (v/v), ajusté à pH 6,0

B) (2,5 g/l) NaH<sub>2</sub>PO<sub>4</sub>/CH<sub>3</sub>CN, 30:70 (v/v), ajusté à pH 6,0

Mode d'élution : gradient linéaire de 5 à 60 % de B dans A en 40 mn

débit : 1,8 ml/mn

détecteur UV : 254 nm

étalon interne : antibiotique A 42867 (R<sub>t</sub> = 8,45 mn)

E) Analyse élémentaire montrant la présence d'un atome de chlore par molécule

F) Analyse NOESYPM RMN-<sup>1</sup>H 2D, montrant que l'atome de chlore moléculaire se trouve sur le cycle 2 et non sur le cycle 6, sur la base de l'effet Nucléaire Overhauser (couplage dipolaire dans l'espace) et du couplage scalaire sur la liaison chimique, et plus précisément :

a) sur la base du couplage scalaire, le proton 2b (voir formule I) ne montre qu'un couplage méta avec le proton 2f mais pas de couplage ortho ; (le proton 2b se trouve sur le cycle 2 et non sur le cycle 6, sur la base du couplage dipolaire suivant :

2b → z'<sub>2</sub> ; 2b → (z<sub>2</sub>)OH ; (z<sub>2</sub>)OH → z'<sub>2</sub> ; z'<sub>2</sub> → x<sub>2</sub>)

b) sur la base du couplage scalaire, le proton 6b (voir formule I) montre un couplage ortho avec le proton 6c (couplage dipolaire 6b → 6c ; z<sub>6</sub> → 6b ; z<sub>6</sub> → x<sub>6</sub>).

G) Un spectre de masse par bombardement atomique rapide (FAB), avec M + H<sup>+</sup> à environ 1251 (les pics ioniques des agrégats théoriques, pour la formule C<sub>60</sub>H<sub>64</sub>N<sub>9</sub>O<sub>15</sub>Cl + H, vont de 1250 à 1255, valeur moyenne 1251,68). Le spectre FAB est obtenu avec un appareil VG 70-250, le gaz de bombardement étant le Xénon ; énergie du faisceau : 8 keV ; tension d'accélération 6 kV ; matrice thioglycérol-glycérol, 2:1.

H) Fonctions acides et basiques pouvant former des sels.

3. Procédé pour préparer un composé selon la revendication 1 ou 2, qui comprend le fait de faire réagir un antibiotique A 42867, ayant les caractéristiques suivantes :

A) Spectre d'absorption UV, qui présente les maxima d'absorption suivants :

	Lambda max (nm)
a) HCl 0,1N	282
b) eau	282
c) tampon phosphate pH 7,4	282
d) tampon phosphate pH 9	282 305 (épaulement)
e) KOH 0,1N	305 265 (épaulement)

B) Spectre d'absorption infra-rouge, qui présente les maxima d'absorption suivants (cm<sup>-1</sup>) :

3700-3100, 3000-2800 (nujol) ; 1650 ; 1580 ; 1460 (nujol) ; 1375 (nujol) ; 1300 ; 1235 ; 1210 ; 1160 ; 1130 ; 1060 ; 1025 ; 1000 ; 970 ; 840 ; 790-700 ; 720 (nujol)

C) Spectre RMN-<sup>1</sup>H qui présente les groupes de signaux suivants (en ppm) à 500 MHz, enregistré dans du DMSO-d<sub>6</sub> (hexadéutériodiméthylsulfoxyde), la référence interne étant le TMS (0,00 ppm), (delta = ppm) :

d<sub>1</sub> 0,90 ; R<sub>6</sub> 1,06 ; V<sub>6</sub> 1,23 ; V<sub>3</sub> 1,52 ; c<sub>1</sub> 1,62 ; b<sub>1</sub> 1,76 ; V<sub>2</sub>,V<sub>2</sub>'-2,30 ; a<sub>1</sub>(N-CH<sub>3</sub>) 2,38 ; R<sub>4</sub> 3,12 ; x<sub>1</sub>,R<sub>3</sub>,V<sub>4</sub> 3,10-3,50 ; V<sub>5</sub> 3,60 ; R<sub>2</sub> 3,79 ; x<sub>6</sub>,R<sub>5</sub> 4,22 ; x<sub>7</sub> 4,51 ; x<sub>5</sub> 4,75 ; V<sub>1</sub> 4,88 ; R<sub>1</sub> 4,96 ; x<sub>2</sub> 5,02 ; x<sub>3</sub>,z<sub>6</sub> 5,12 ; z'<sub>2</sub> 5,22 ; 4f 5,33 ; 4b 5,53 ; z<sub>2</sub>(OH) 5,88 ; 7f 6,23 ; x<sub>4</sub> 6,34 ; 7d 6,41 ; h 6,62 ; 5c,w<sub>3</sub> 6,76 ; 5f 6,84 ; 6c 7,12 ; 5b 7,15 ; 2b 7,26 ; w<sub>6</sub>,h 7,32 ; 6b 7,50 ; w<sub>4</sub> 8,15 ; w<sub>7</sub> 8,45 ; 7c 9,10 ; 5d,e<sub>1</sub> 9,32 ; 7e 9,39 ;

D) Temps de rétention ( $R_t$ ) égal à 0,665 par rapport à la Vancomycine.HCl (Vancocin, Eli Lilly,  $R_t = 9,96$  mn), l'analyse étant effectuée par CLHP en phase inversée dans les conditions suivantes :

colonne : Ultrasphère ODS (5 micromètres) Altex (Beckman), diamètre intérieur 4,6 mm x 250 mm

pré-colonne : Brownlee Labs RP 18 (5 micromètres)

éluant A :

$\text{CH}_3\text{CN} : 2 \%$   
 $(2,5 \text{ g/l}) \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O} : 98 \%$  } ajusté à pH 6,0

éluant B :

$\text{CH}_3\text{CN} : 70 \%$   
 $(2,5 \text{ g/l}) \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O} : 30 \%$  } ajusté à pH 6,0

élution : gradient linéaire de 5 à 60 % de l'éluant B dans l'éluant A, en 40 mn

débit : 1,6 ml/mn

détecteur UV : 254 nm

étalon interne : Vancomycine.HCl ( $R_t = 9,96$  mn) (Vancocin, Eli Lilly)

E) Analyse élémentaire, après séchage préalable de l'échantillon à environ 140 °C sous atmosphère inerte, qui indique la composition approximative suivante en pourcentage (moyenne) : carbone 53,3 % ; hydrogène 5,9 % ; azote 7,85 % ; chlore (total) 4,41 % ; chlore (ionique) 2,22 %. Résidu minéral à 900 °C dans l'air : 0,875 %.

F) Profil de titrage acide-base dans l'eau après titrage avec une solution aqueuse 0,05N de KOH, effectué sur un échantillon préalablement additionné d'un excès de HCl en solution aqueuse, qui présente des valeurs de pKa de 3,2, 7,1 et 8,3.

G)  $R_t$  de 0,56 dans le système chromatographique suivant :

un mélange 70:30, ajusté à pH 6,0, de NaCl en solution aqueuse (87,5 g/l)/ $\text{NaH}_2\text{PO}_4$  en solution aqueuse (0,5 g/l) et d'acétonitrile

utilisation de plaques de gel de silice silanisé en phase inversée (RA-18 F<sub>254</sub>).

Visualisation :

- lumière UV à 254 nm

- couleur jaune avec le réactif de Pauly, c'est-à-dire l'acide sulfanilique diazoté (J. Chromatog. 20, 171 (1965), Z. Physiol. Chem. 292, 99, (1953))

- bio-autographie avec *B. subtilis* ATCC 6633 sur un milieu minimal de Davis.

H) Poids moléculaire d'environ 1559, d'après un spectre de masse par bombardement atomique rapide (FAB-MS) présentant le pic  $M + H^+$  à 1560.

4. Procédé selon la revendication 1, 2 ou 3, dans lequel l'acide fort est l'acide chlorhydrique.

5. Procédé selon la revendication 1, 2, 3 ou 4, dans lequel le solvant est le diméthylsulfoxyde ou le diméthylformamide.

6. Procédé selon la revendication 1, 2, 3, 4 ou 5, dans lequel la température de réaction est comprise entre 80 et 110 °C.

7. Utilisation d'un composé selon la revendication 1 ou 2 pour préparer un médicament destiné à être utilisé comme antibiotique.





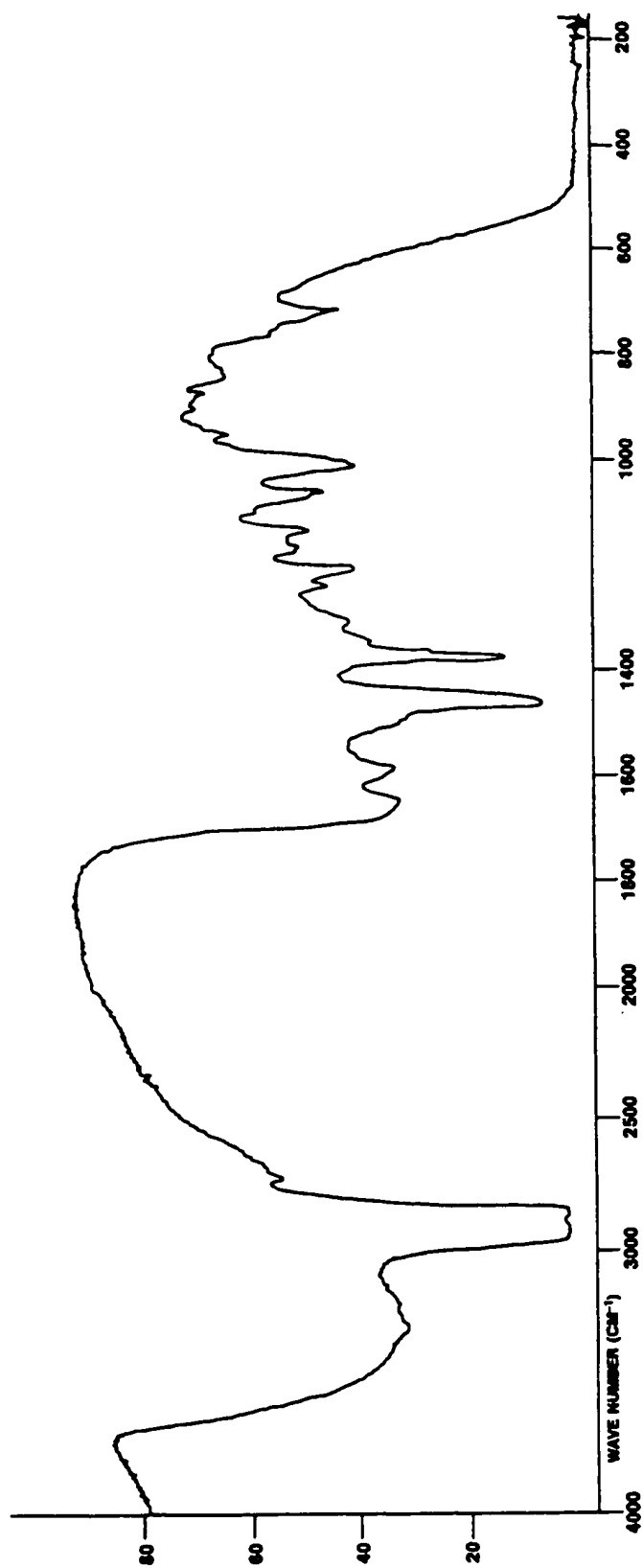


FIG. 2

FIG. 3

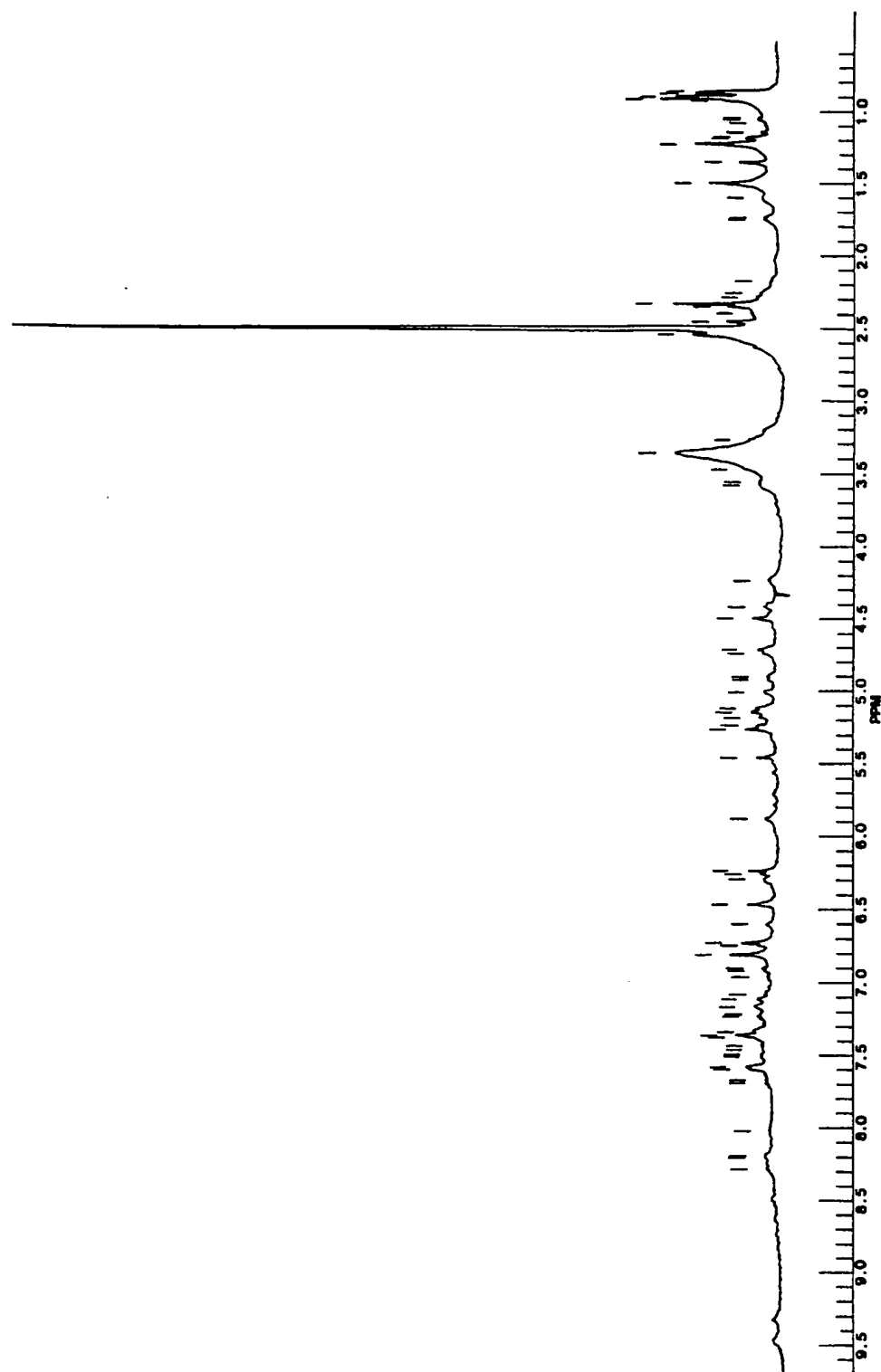


FIG. 4

